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Auditory Activity
in Centrifugal and Centripetal
Cochlear Fibres in Cat

A Study of A Feedback System

BY

JÖRGEN FEX

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AUDITORY ACTIVITY
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COCHLEAR FIBRES IN CAT
A STUDY OF A FEEDBACK SYSTEM

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INTRODUCTION

A system for direct nervous control of the inner ear activity was discovered by RASMUSSEN (1946). Using the Marchi degeneration method he could trace a group of fibres from the superior olivary complex to the vestibulo-cochlear anastomosis in the contralateral inner ear. In a later study RASMUSSEN (1953) could follow these crossed olivo-cochlear fibres to a region peripheral of the spiral ganglion of the cochlea.

A multitude of recent experimental data suggest that the input from many afferent systems are controlled by centrifugal nervous pathways and that centrifugal control may be a general principle of action of the central nervous system. For discussion and literature of such systems see GRANIT (1955) and HAGBARTH (1960). The most penetrating experimental studies of a centrifugal control system are those of GRANIT and his co-workers over the efferent gamma control of the muscle spindles (see GRANIT 1955).

Available data on the different systems controlling sensory input indicate that they may fulfill at least two different functions. Studies by HERNÁNDEZ-PEÓN and co-workers (for recent findings and reference to earlier literature see BACH-Y-RITA, BRUST-CARMONA, PENALOZA-ROJAS and HERNÁNDEZ-PEÓN 1961) suggest that centrifugal control may act as a gating mechanism, letting through the sensory input of highest significance in any actual situation and suppressing sensory input of secondary importance. Another centrifugal control function is found in self-regulating systems where the sensory input is controlled through a feedback loop activated by the sense organ itself. A simple example of such a regulatory device is the pupil reflex to light which recently has been analyzed in terms of modern servotechnique (STARK 1959). The middle ear muscles seem to have a similar function of controlling the amount of stimulating energy to which the receptor is subjected.

The function and the significance of the efferent system of the crossed olivo-cochlear fibres of RASMUSSEN remain largely obscure. Some effects of direct electrical stimulation of these fibres have been studied, but nothing is known about how and from where they are activated. GALAMBOS (1946) showed that electrical stimulation of the crossed olivo-cochlear fibres in the floor of the fourth ventricle depressed or abolished the action potential component of the round window response to a click. GALAMBOS' findings were confirmed by FEX (1959) who showed that such electrical stimulation also increased the size of the cochlear microphonic potentials at the round window. RUBEN and SEKULA (1960) stimulated the crossed olivo-cochlear fibres similarly and found it possible to abolish the response of the auditory cortex to a click

when relatively weak electrical stimuli were applied, without changing the action potential at the round window. At higher stimulus strength the round window action potential was also abolished. DESMEDT and MONACO (1960) showed that the effect on the action potential at the round window from electrical stimulation of the crossed olivo-cochlear fibres was blocked by strychnine and (DESMEDT and MONACO 1961) that the cochlear microphonic increase in response to such stimulation was also blocked by strychnine.

The studies referred to seem to indicate that the crossed olivo-cochlear fibres have an inhibitory effect upon auditory input at the inner ear level perhaps on "certain key fibers in the eighth nerve essential for a cortical-evoked potential" (RUBEN and SEKULA 1960). The suppression of the synchronous action potential component of the round window response to sound cannot, however, be regarded as evidence that only inhibitory processes are at play. It may well represent a summative effect of both inhibitory and facilitatory processes. This question had to be approached by microelectrode analysis at the level of the primary auditory neurones (cf. HAGBARTH and FEX 1959, for a discussion of the necessity of supplementing macroelectrode studies by microelectrode investigations).

Since according to current theories (cf. DAVIS 1961) the relation between cochlear microphonics and action potential at the round window is that of receptor potential to generated neuronal activity, the above mentioned findings of GALAMBOS (1956) and of FEX (1959) seemed to merit further analysis, especially with regard to the site of action of the efferent system.

In the present work I have approached the following problems concerning the function of the crossed olivo-cochlear fibres 1) are these fibres acoustically activated and could they be found to participate in a feedback control, 2) how is activity of the primary auditory neurons influenced by electrical stimulation of the crossed olivo-cochlear fibres and 3) where is the site of action of these fibres. Each of the three main problems will below be treated in a separate Chapter. Thus Chapter I concerns a single fibre analysis of the efferent fibres. It will be shown that such fibres are activated by sound, In the next two Chapters the effects of these efferents are further studied. In Chapter II this was done by recording from single primary auditory fibres. These were identified according to a histological criterion. Only inhibitory effects were found. In Chapter III the action of crossed olivo-cochlear fibres on some of the electrical events of the inner ear were studied in an attempt to gain some insight into the site of action of these fibres. Earlier observations on the action potential were confirmed and the observations on the cochlear microphonic were extended. It will also be shown that the resting potential at the round window was increased by stimulation of the olivo-cochlear bundle.

CHAPTER I

SINGLE FIBRE ANALYSIS OF CROSSED EFFERENT FIBRES

In this series of experiments the response of the crossed olivo-cochlear fibres to sound has been analyzed, as part of the study of the function of these fibres.

To enable better understanding of the procedures to be described it seems pertinent here to give a brief summary of RASMUSSEN's (1946, 1953, 1960) histological results obtained from cats, rats and opossums (1946) and from cats (1953, 1960).

The origin of the crossed olivo-cochlear fibres is confined chiefly to an area situated medial to the accessory olive and dorsal to the nucleus of the trapezoid body. In this region small multipolar cells known as the retro-olivary group are found. These cells are morphologically of the visceral efferent type; their dendrites intermingle with the fibrous plexus of the superior olivary complex and their axons are directed dorsally. The crossed olivo-cochlear fibres rise through the brainstem to the floor of the fourth ventricle. Before crossing the raphe they collect into a compact bundle beneath the facial genu. The level of decussation is localized to the rostral border of the facial genu. After crossing, the fibres fan out but reconverge near the lateral side of the facial root. At that point a few fibres enter in the medial angle of the medial vestibular nucleus and terminate about small cells in this nucleus. The remaining fibres course laterally as a compact bundle to the dorsal border of the descending root of the fifth nerve where the crossed bundle joins the incoming vestibular root. When leaving the ventrolateral margin of the fifth root the crossed olivo-cochlear bundle is broken up into three or more fascicles by fibres of the lateral trapezoid body and its latero-caudad course is changed abruptly. At the point of emergence from the medulla the crossed olivo-cochlear fibres have a course parallel with that of the vestibular and facial roots. At the level of the pia ring the crossed olivo-cochlear fibres are found to be located near the ventral surface of the vestibular root and can there be followed between the two divisions of the vestibular nerve. More distally they are covered ventrally by the inferior vestibular division, becoming more deeply situated in the vestibular nerve upon coursing peripherally. When passing through the ganglion associated with the saccular nerve the scattered fibres tend to converge to the ventral extremity of the ganglion. At that point they leave the

vestibular nerve to accompany the cochlear nerve as the vestibulo-cochlear anastomosis of OORT (1918). The crossed olivo-cochlear fibres join the cochlear nerve between the basal and second turn entering into a spiral formation at the external margin of the spiral ganglion and from there fibres are distributed peripherally throughout all turns towards the organ of Corti. The fibres were found to extend beyond the spiral ganglion but could not be followed beyond a delicate spiral plexus situated near the margin of the osseous spiral lamina. This was considered by RASMUSSEN as perhaps due to technical difficulties.

RASMUSSEN (1960) added information about a connection of the crossed olivo-cochlear bundle with the ventral cochlear nucleus. Fine fibres leave the main crossed olivo-cochlear bundle to traverse the glial portion of the vestibular root in a dorsolateral direction. Most of the fibres enter and terminate within the superficial cell layer of the ventral cochlear nucleus and the remainder enters the nucleus proper in a diffuse fashion. The precise terminations of these fibres were not determined.

The presence of an uncrossed olivo-cochlear tract (RASMUSSEN 1960) has also been demonstrated. Its fibres emanate from the S-shaped olivary segment, to pass dorsal and lateral to the ascending limb of the crossed component. The uncrossed olivo-cochlear fibres join the crossed olivo-cochlear fibres from the opposite side lateral to the facial root. In the same study he concluded that the total number of crossed olivo-cochlear fibres in the cat is around 500 and that the uncrossed olivo-cochlear tract has a fibre population about a fourth of this number. A short description of the synaptic connections of the two fibre systems was presented. The nucleus of origin of the homolateral efferents receives exclusively uncrossed connections from the cochlear nucleus, while the cell groups associated with the origin of the crossed olivo-cochlear fibres receive afferent connections, predominantly, if not entirely, from the cochlear nucleus of the opposite side. "In other words, the composite bundle of one side arises from nuclei that are connected with cochlear nucleus on the same side as that from which the bundle leaves the brain."

Technique and Procedure

Selection of experimental animals

The experiments in this series were carried out on 47 cats, weighing between 1.5 and 2.5 kg. The animals were carefully inspected and chosen only if a) their behaviour indicated normal hearing, b) they were free from signs of respiratory infections, c) they under otoscopic inspection showed a normal light reflex from the tympanic membrane, d) their outer ears were free from

mite (*Otodectes cynotis*). Mites may cause pathological changes of the tympanic membrane and thus impairment of the cat's hearing. Only one cat, 28.09.61, infected with this parasite has been used. During the preparation of the animal, any pathological changes found in the middle ear or the inner ear under the dissecting microscope were used as a basis for elimination.

The preparation of the animal for the experiment

Under ether anaesthesia the carotid arteries were ligated and the trachea and one of the femoral veins were cannulated. When a femoral vein cannula had been inserted the administration of the ether was stopped, as a rule not more than 15 min after its initiation, and a short-lasting barbiturate, Thiogenal ("E. Merck"), was given in a dose of 10—20 mg/kg body weight. A long incision was made in the midline over the skull, the skin and the muscles were reflected so that the external acoustic meatus could easily be exposed on both sides. The cat's head was then firmly attached to the head holder, a modified Horsley-Clarke instrument of the Labtronic's model. Holes were drilled in the most lateral part of each temporal bone and in these holes pointed metal pins were inserted which served the same function as conventional ear pins. The eye-holder was adjusted to match the height of the pins in the parietal bones. A large opening was made in the parietal bone to the left of the midline and the cat was decerebrated precollicularly by suction.

Using a temporal approach the left tympanic bulla was exposed and opened, the bony ring enclosing the tympanic membrane was removed and the left outer ear reflected. Subsequent stages in the dissections were carried out with the aid of a binocular operating microscope (Zeiss Epiteknoskop), which during the latter part of the study had the Zeiss motordriven control for focusing, operated by a foot switch. The ossicles were removed but the tympanic tensor muscle was left. The reflex contraction of this muscle to sound was used as a gross test of the function of the contralateral (right) ear during the experiment. A small hook was inserted in the oval window towards the round window and the bony part between these openings removed by pulling the hook. This opened the vestibule and the opening was widely enlarged upwards with a dental drill. The floor and the medial wall of the vestibule were thus exposed and parts of these structures were removed with a fine drill. The inferior part of the vestibular nerve was now accessible and was divided with finely pointed watchmaker's forceps. To cite RASMUSSEN (1946, p. 170: "Under a dissection microscope Oort's anastomosis comes to view upon elevating the inferior division of the vestibular nerve from the groove formed by the basal and middle turns of the cochlear nerve. It is also observed that the main bundle courses in a bed of connective tissue and

accompanies blood vessels near the bottom of the above mentioned groove and passes at right angles to the fibres of the basal turn (Fig. 5)." The bed of connective tissue and the blood vessels mentioned by RASMUSSEN had to be reflected since they completely obscure the vestibulo-cochlear anastomosis in the living animal. This was by far the most delicate part of the preparation. The connective tissue was visualized under a magnification of 60 times and torn by the pair of finely pointed forceps. Suction was applied with glass tubes drawn to tip diameters of 0.2—0.5 mm and flamed. In spite of every precaution blood vessels were often torn with the connective tissue. At this stage of the preparation even the smallest blood clot formed in the operative field could ruin the preparation. In order to prevent formation of blood clots outside the vessels the preparation was repeatedly flushed with Ringer solution to which Heparin ("Vitrum") 3 I. U./ml had been added. This did not prevent closure of torn blood vessels, although bleeding took longer time to stop than it would have done if external blood clot formation had been allowed. This "Heparin flusing technique" proved to be of great importance for a successful preparation.

The cerebellum was exposed and part of it removed over the fourth ventricle. Under visual control stimulation electrodes were placed between the facial genua in the floor of the fourth ventricle. The craniotomies and the drillholes were closed with dental cement to minimize pulsations of the brain. The right pinna was amputated close to the bone ring enclosing the tympanic membrane, so that the reflected ear would not attenuate the sound stimuli to be presented.

In a series of 20 animals stimulating electrodes were placed in the cochlear nerve peripheral to its basal turn. Any bleeding that occurred during the enlargement of the opening of the vestibular floor was controlled by gel-foam (Spongostan, "Ferrosan"). Special care was taken not to contaminate the region of the vestibulo-cochlear anastomosis with Spongostan.

To prevent elevation of blood pressure, Thiogenal in doses of 10—20 mg/kg body weight was given i. v. just before the preparation of the left inner ear and before the neck muscles were detached. Thiogenal was sometimes used at the end of the experiment to decrease movements in lively animals. Flaxedil ("May & Baker") in doses of 8—10 mg/kg body weight was occasionally used for the same purpose and the cat was then artificially respiration.

Electrical stimulation

The stimulator used had an output resistance of 1 000 ohms and delivered square wave pulses, but the pulse form was distorted by the insulation transformer. Pulses of 0.6 msec were used either as single shocks or in trains

of tetanic bursts. The burst duration, the pulse frequency and the pulse amplitude could be controlled independently of each other. Stimulating current was not measured.

For stimulation of the left cochlear nerve peripheral to its basal turn, two insulated copper wires with a diameter of $40\ \mu$ were used, the bared tips being 0.5 mm long and 0.5 mm apart from each other and from the recording site in the vestibulo-cochlear anastomosis.

For stimulation of the region where the olivo-cochlear fibres cross in the floor of the fourth ventricle, electrodes were used which consisted of two enamelled platinum-iridium wires. The diameter of each wire was 0.2 mm, the interelectrode distance was 0.7—1.0 mm and the uninsulated 1 mm long pointed tips protruded from cuffs of polyethylene tubing that served as stoppers at the floor surface.

In 5 animals histologic sections were studied of the stimulation site between the facial genua in the floor of the fourth ventricle. Electrode tracts were found in, or close to, crossing bundles that seemed to fit the description given by RASMUSSEN (1946) for the crossed olivo-cochlear bundles.

Acoustic stimulation

Acoustic stimulation was carried out with a sine wave generator ("Philips" Model GM 2305 B). Its attenuator was used in either of two positions, separated by a step of the order of 40 db. Finer gradations of the applied sound pressure at this part of the acoustic system were achieved with the aid of a separate attenuator provided with two series coupled divisions, one with 10 steps of 6 db each, the other with 10 3 db steps. The connection between the tone generator and the attenuator was plugged and unplugged by hand when longer pips independent of the sweep circuit were used. This technique was used during a great part of many of the experiments, since it was discovered that the transients presented no serious problem. The output of the tone generator was fed into an electronic gate (Brüel & Kjaer type F1) when short pips were wanted. The gating signal came from the stimulator and was synchronous with the signal that triggered the sweep to the oscilloscope. Time of rise and decay as well as duration and amplitude of the pips could be varied independently of each other. Pips from this gate were used for latency determinations and were presented to the cat through a hearing aid receiver that was mounted in one end of a 9 mm long tube; the other end faced the tympanic membrane at a distance of 2—3 mm. The electronic gate could be used only within a comparatively narrow sound pressure range and for many measurements another system was employed. Occasionally acoustic "clicks" were produced by passing the short impulse, used for starting the stimulator,

through a power amplifier connected to the attenuator. The characteristics of the "click" were not determined.

The output from the attenuator was presented in an open field through a dynamic loudspeaker ("Philips" 7910) mounted in a 10 l closed back cabinet. The loudspeaker was placed 30 cm from the exposed right tympanic membrane with its axis directed towards the membrane.

The frequency response for the loudspeaker system was flat within ± 6 db between 200—5 000 c/s and showed a few dips of the order of 15 db between 5 000 and 7 000 c/s. The magnitude and location of these dips differed with different positions of the loudspeaker within experimental conditions. Above 7 000 c/s there was a downward slope of the order of 30 db/octave. The loudspeaker's maximal output was 115 db SPL (SPL = Sound Pressure Level relative 0.0002 dyn/cm²). These figures were obtained under experimental conditions with a Brüel condenser microphone (No. 4133) mounted 5 mm in front of the upper rim of the tympanic membrane to give a "parallel sound incidence". A few measurements of absolute sound pressures of stimuli given under experiments were done with the same instrument.

With a "Rudmose" sound Analyzer (model RA 100) octave-band analyses were made over intervals of one minute under experimental conditions. The following figures for noise levels in the room were obtained: 47 db, 50 db, 40 db, 35 db, 27 db and less than 22 db SPL in respective frequency band's 37.5—75 c/s, 75—150 c/s, 150—300 c/s, 300—600 c/s, 600—1 200 c/s, and the three bands 1 200—2 400 c/s, 2 400—4 800 c/s and 4 800—9 600 c/s. The figures for the frequencies 600—9 000 c/s were not high, but the background noise may have influenced the results.

Recording technique

Capillary microelectrodes filled with 4 M NaCl and having a resistance, as measured in the preparation or in Ringer solution, of 20—40 megohms were used for recording. The electrode, mounted on a micromanipulator fixed to the Horsley-Clarke instrument, was connected by a cathode follower to an AC-coupled differential amplifier. The input of the cathode follower was 2.5 pf and the capacity of the micropipette was of the order of 5 pf. The reference electrode was an Ag-AgCl wire placed on the rim of the opened round window. The grounding Ag-AgCl wire was put on the left temporal muscle with a piece of cotton soaked in Ringer solution. The differential amplifier fed a split beam cathode ray tube, a pair of headphones and one of the two channels of a tape recorder ("Revox-Stereo", Model D 36). The neuronal activity from the preparation was monitored acoustically with the headphones and visually with the cathode ray tube. On a few occasions the

cathode ray beam was photographed but as a rule the experiment was recorded on the tape recorder. The second channel of the tape recorder was used for recording protocols, triggering signals, and the electrical equivalent of the stimuli used. After the experiment the tape was played back into the cathode ray oscilloscope using one beam for the recorded neurophysiological events and the other for the recorded stimuli or for time calibration. Sweeps were thus displayed and photographed having the same synchrony of events as in the original experimental setting. This technique was advantageous in many ways. There was no time lost during the actual experiment in deciding exactly how to display the events on the cathode ray oscilloscope. The decision was made during repeated playbacks after the experiment, when the tape recorder's loudspeaker provided acoustical monitoring of the recorded signals or gave the protocol of the experiment. Occasionally, it was found very convenient during the experiment to play back the tape on a Tektronix Type 522 Oscilloscope to check some finding and this could thus be done faster than if conventional photographic procedures had been involved. The tape recorder was started and stopped by a foot switch while the input to the second channel was controlled by a manual switch. When sweeps were played back and triggered from the tape's synchronous signals the first 0.1 msec of the sweeps were lost. The frequency response characteristics of the tape recorder were such that no conclusions could be drawn from the shape of the potentials recorded. No attempts were made systematically to record absolute size, shape or polarity of spikes.

Stability of the preparation

Recording from single neuronal elements requires very stable conditions. In an attempt to minimize the pulsations of the brain, all openings made in the skull were closed with dental cement, excluding of course the one for the left vestibulo-cochlear anastomosis. As a further attempt to immobilize the left eighth nerve the intracranial part of the nerve and the region of the cochlear nuclei were covered by a firm gel of agar-agar in Ringer solution. However, the stabilizing effect of this procedure was doubtful. A somewhat more effective method was used in a large group of cats. The part of the cerebellum overlying the intracranial part of the eighth nerve and the region of the cochlear nuclei was sucked away. Three or four insect pins with a diameter of 0.15 mm were inserted under visual control through a hole drilled in the temporal bone and into the intracranial part of the eighth nerve and the region of the cochlear nuclei until their tips were firmly anchored in the underlying bone. In 20 cats the left cochlear nerve was electrically stimulated and so the eighth nerve and the region of the cochlear nuclei had to be

kept intact. It was assumed that the critical fact was that the skull was not totally closed and then the size of the hole for the vestibulo-cochlear preparation in the vestibule did not seem to matter. Another main factor that would cause instability was pulsation of blood vessels close to the vestibulo-cochlear anastomosis. Often the most apical fascicle of the anastomosis was immediately overlying a pulsating blood vessel and these structures could not be deflected from each other.

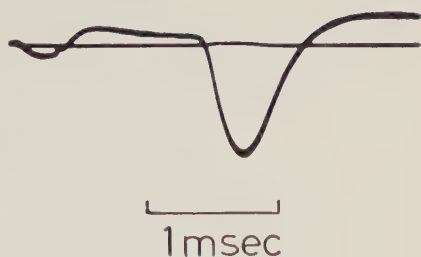
Even in the most stable preparations only few units could be recorded from for as long as 15 min. Many units were kept for only 1—5 min, and many for just a few seconds.

Edema of the brain stem tended to herniate the intracranial part of the eighth nerve through the internal meatus with consequent interference with the blood supply of the preparation. Edema was one of the big difficulties encountered with these preparations until urea administration became the routine. A 30 % urea solution with 10 % inert sugar proved highly effective in preventing brain edema (see JAVID and SETTLAGE 1956, JAVID 1958) when given in three to four doses of 0.30—0.40 g/kg body weight at intervals of one to two hours.

Control of the topography and histology of the fascicles of the vestibulo-cochlear anastomosis

The vestibulo-cochlear anastomosis was carefully dissected following each experiment. The region was flushed with a 1 % solution of osmic acid which stained the nervous tissue black, differentiating it from other tissues. At the same time such procedures made the thin fascicles of the vestibulo-cochlear anastomosis less liable to tear when manipulated. It was found in some animals that the most apical fascicle had been hidden behind its neighbouring fascicle during the experiment. The direction of the apical fascicle or fascicles was noted whenever it differed from what had been found to be the rule in preparations studied preceding this series of experiments. In at least two of the cats a relatively large part of the apical fascicle took a radial course already 2 mm distal to the anastomosis, which was exceptional. Histological sections of the osmium stained vestibulo-cochlear anastomosis were studied in 7 animals. The 3—5 fascicles varied in width between 50 and 200 μ and were tightly packed with nerve fibres while no nerve cell bodies were identified. The fibre diameters ranged from 1—5 μ with most of them being from 3—5 μ . These figures were arrived at by measuring fibres using a measuring ocular. The figures thus represent an approximation, but since they agreed with RASMUSSEN's findings (1946) that the main part of the vestibulo-cochlear anastomosis consists of crossed olivo-cochlear fibres with a diameter of 3—5 μ , there seemed no reason for having recourse to more elaborate procedures.

Fig. 1. Five superimposed action potentials recorded from a single crossed olivo-cochlear fibre in the vestibulo-cochlear anastomosis. Single shocks were applied to the crossed olivo-cochlear bundle in the floor of the fourth ventricle about 15 mm from the recording site. Negativity is upwards. The initial, small, positive deflection is the shock artifact. The latency from the beginning of the shock to the inflection point of the initial positive phase of the action potential was 1.6 msec. Cat 20.06.61.



Conduction of the experiment

If they could be separated at all from each other, the 3—5 separate fascicles of the vestibulo-cochlear anastomosis in the living animal were seen as 50—200 μ wide strands of nervous tissue, often poorly outlined against the background. Through the approximate alignment of the shaft of the micropipette with the optical axis of the binocular operating microscope it sometimes was possible to control the direction of the microelectrode so that it could be placed in any division of the vestibulo-cochlear anastomosis, the limiting factor being the poor visibility of the anatomical structures. When the micropipette tip travelled the last few hundred microns towards the vestibulo-cochlear anastomosis the micromanipulator was controlled with the experimenter's left hand, his right hand kept the vestibulo-cochlear anastomosis free from fluid by micro suction while the microscope focusing was controlled with his left foot.

The physical contact between the microelectrode tip and the vestibulo-cochlear anastomosis was signaled by a change in the noise from the monitoring headphones. Immediately following contact a temporary 22 megohm grid leak to ground was removed from the cathode follower, the microscope was turned away and a few drops of Heparin in Ringer solution were applied to the recording site to minimize the risk for blood clot formation (see p. 10). Single shocks at the rate of 1—4 shocks/sec were applied to the site where the olivo-cochlear bundles cross in the floor of the fourth ventricle. The shocks were synchronous with the signal triggering the sweep on the monitoring oscilloscope. The tip of the microelectrode was slowly advanced. As a rule, a change of noise indicated that the tip was in contact with a nerve axon. The micromanipulator was now handled with great care and as a rule an evoked potential soon appeared on the monitoring oscilloscope. The tape recorder was immediately started and sometimes the potential was photographed from the monitoring oscilloscope (Fig. 1). For several of the fibres,

recording conditions were such that nothing but these electrically evoked potentials could be taped, perhaps with the addition of some activity aroused by the experimenter's voice, a whistle or some other sound stimulus not presented through the loudspeaker. Whenever possible, a unit identified by single shocks as a crossed olivo-cochlear fibre was studied with acoustical stimuli from the loudspeaker and/or with electrical stimuli applied to the left cochlear nerve. The fibre activity was monitored through both the headphones and the cathode ray oscilloscope and was recorded on tape. Particulars for the different experimental situations will be given in the description of the results.

The stimulus strength used, as expressed in voltage output of the stimulator, varied between 0.8 V and 8 V, 2 V being the strength generally used. If single shocks of 4–8 V to the cochlear nerve did not activate a fibre, this fibre was considered unexcitable by single shocks.

There were no systematically recorded observations pertaining to the general condition of the animal. In some experiments it was noticed that there was a prompt reflex contraction of the homolateral tympanic muscle to moderate sound presented to the contralateral ear, even when very strong sounds were needed to evoke activity in the fibres studied. This tympanic muscle reflex was taken to indicate that the right ear and the auditory pathways of the cat were still in good condition. The activity of the right inner ear was not monitored in any direct way.

Results

Criterion and introduction

Once it had been established that microelectrodes of comparatively high resistance, *i. e.* with very fine tips, had to be used, single unit activity was recorded from every cat in which the vestibulo-cochlear anastomosis was probed with a microelectrode. Single unit activity in the anastomosis was driven by electrical stimulation of the floor of the fourth ventricle between the facial genua. The site, where the crossed olivo-cochlear bundles intersect (RASMUSSEN 1946) in the midline between the rostral part of the facial colliculi was as a rule easily visualized so that the stimulating electrodes could be correctly placed. For the identification of a fibre in the vestibulo-cochlear anastomosis as a crossed olivo-cochlear fibre it was considered a necessary and an adequate criterion that it could be electrically driven from the floor of the fourth ventricle (see Discussion, p. 52).

In the 47 cats of this experimental series 505 crossed olivo-cochlear fibres were found. Many of these were lost before they could be adequately tested,

but 308 fibres were activated by acoustical stimulation of the contralateral ear or by electrical stimulation of the homolateral auditory nerve. There were only 9 acoustically activated fibres in 7 cats that failed to meet the criterion for crossed olivo-cochlear fibres. The high incidence of crossed olivo-cochlear fibres encountered, 505 out of 514, seems surprising considering that the fibre population of the homolateral component in the vestibulo-cochlear anastomosis was judged by RASMUSSEN (1960) to be approximately one fourth that of the crossed component. The explanation may lie in the fact that the crossed olivo-cochlear fibres were repetitively stimulated in the floor of the fourth ventricle, which guided the probing with the microelectrode and biased the finding of crossed olivo-cochlear fibres in the vestibulo-cochlear anastomosis.

When a fibre had been identified as belonging to the crossed olivo-cochlear bundle the presence or absence of resting activity was noted, its response to acoustic stimuli was studied, and the following questions were asked: 1) Is there a graded response to sound stimuli. 2) Can a threshold to sound stimuli at some particular frequency be well defined. 3) Is the threshold very low as measured in db SPL. 4) Is there a best frequency at which the threshold for stimulation of the fibre is lower than for any other frequency. 5) Does the response to sound or to electrical stimulation of the cochlear nerve occur with a reproducible latency. A positive answer to any one of these questions would indicate that auditory stimuli are adequate for activation of the crossed olivo-cochlear fibres.

Resting activity

Thirty-five fibres out of 308 activated crossed olivo-cochlear fibres showed resting activity. This activity, which never occurred in bursts, had a frequency of 1—10 imp/sec except in cat 28.07.61 in which 9 out of 13 crossed olivo-cochlear fibres had resting activity consisting of 10—20 imp/sec. It may be significant that this cat was more lively than any other in the series. The question as to a possible correlation between spontaneous activity and low threshold or high impulse frequency in the response to acoustical or electrical stimulation of the auditory nerve must be left open. The scarcity of resting activity in a room with a noise level as given on p. 12 was in itself of interest and also had the practical consequence that responses to sound as a rule could be studied without any obscuring background.

Response to sound

A regular firing rate in response to sound stimulation has been a characteristic common to all crossed olivo-cochlear fibres found in these experiments. By

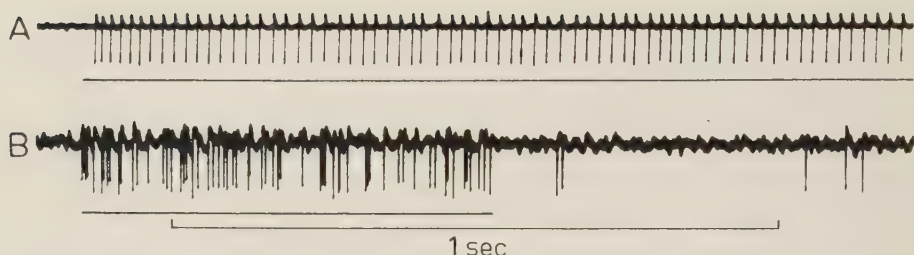


Fig. 2. Responses to tone pips from a single efferent fibre (A) and from a primary auditory fibre (B). Note the regular firing pattern in A in contrast to the irregular pattern in B. The records are from different experiments. The durations of the sound stimuli are indicated by the horizontal bars under the records. Cat 30.07.61. (A). Cat 08.12.61. (B).

contrast, primary as well as secondary auditory neurones often show an irregular pattern (GALAMBOS and DAVIS 1943; KATSUKI, SUMI, UCHIYAMA and WATANABE 1958; ROSE, GALAMBOS and HUGHES 1959). Fig. 2 illustrates this difference: the response to sound is shown for a crossed olivo-cochlear fibre in A and for a primary auditory fibre in B.

Bursts, which are common to the pattern of primary and secondary auditory neurones were as a rule not seen in the crossed olivo-cochlear fibres in response to sound stimuli. Fig. 3 represents the extreme in the way of a phasic component of the response. Less exceptional at the other extreme is the finding shown in Fig. 4 C1, unit 4 from cat 27.10.61, in which the first spike interval was longer than the next few intervals. This kind of initial pattern was as common as the one shown in Fig. 2 A, where the first spike interval was the shortest.

The low firing rate also marked a difference between crossed olivo-cochlear fibres on the one hand and primary and secondary auditory fibres on the other. In these afferent fibres firing rates up to 1 000 imp/sec have been found (GALAMBOS and DAVIS 1943; TASAKI 1954). Fig. 4 illustrating the relationship between stimulus and response, shows 4 fibres that reached firing rates of between 30—50 imp/sec at sound pressures of 60 db relative to their thresholds. For plotting the curves in Fig. 4 the thresholds were determined and the sound pressure was raised first in steps of 3 db and then in steps of 6 db. Long tone pips of 1—2 sec duration or more were used with intervals somewhat shorter or of the same duration. The number of impulses that occurred during the first second of the pips was determined on the filmed playback of the record and plotted against sound pressure expressed in db

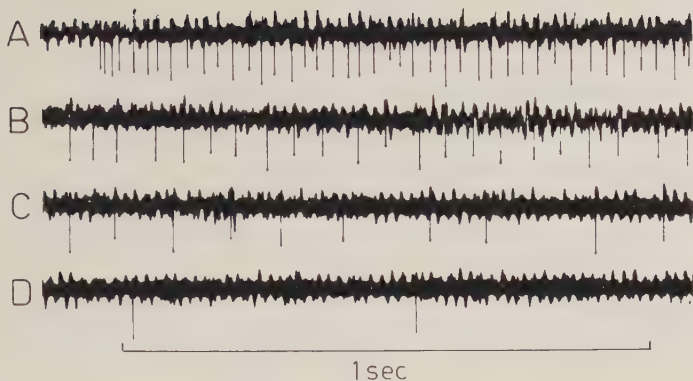


Fig. 3. Response from a single efferent fibre to a continuous tone of its best frequency 2 900 c/s, 30 db above threshold. The tone outlasted the activity which stopped completely after 12 sec; second impulse in record D. Between each of the records there are 2.6 sec intervals. Cat 19.08.61.

relative to the threshold. The firing rate of the fibres of Fig. 4 A had not reached a plateau at 60 db above threshold. Most olivo-cochlear fibres tested in this respect gave graded responses to sound but several of them had a dynamic range of approximately 30 db or less. It is not known whether 60 db is close to the maximum for the dynamic range of crossed olivo-cochlear fibres. Only one fibre, Fig. 4 B, was tested beyond this range and it showed a maximum firing rate at 66 db above threshold.

The firing rates illustrated in Figs. 2—4 are representative, but there were many fibres that could not be stimulated to discharge more than 10—20 imp/sec and a few that responded maximally to sound with only 2—5 imp/sec. Some fibres were stimulated with short, strong tone pips in an effort to make them fire pairs of impulses with short interspike intervals and in Fig. 7 is shown how the spike interval decreases with increase of sound pressure. The extremes found were spike intervals of 4—7 msec (unit 1 cat 24.05.61) and 7 msec (unit 3 cat 28.07.61). The highest firing rate at the beginning of a train of impulses was 9 impulses during 10 msec (unit 3 cat 04.08.61).

In response to loud continuous tones some fibres showed a pronounced decrease in firing rate with time; for the fibre in Fig. 3 the activity stopped after stimulation for 12 sec. The resting activity that was present in some fibres was silenced for several seconds after loud tone pips of long duration. In only one fibre (unit 7 cat 18.06.61) was there discharge of impulses after the end of the sound stimulus. The after-discharge had a duration of more than 3 sec and was observed before and during the effect of Thiogenal.

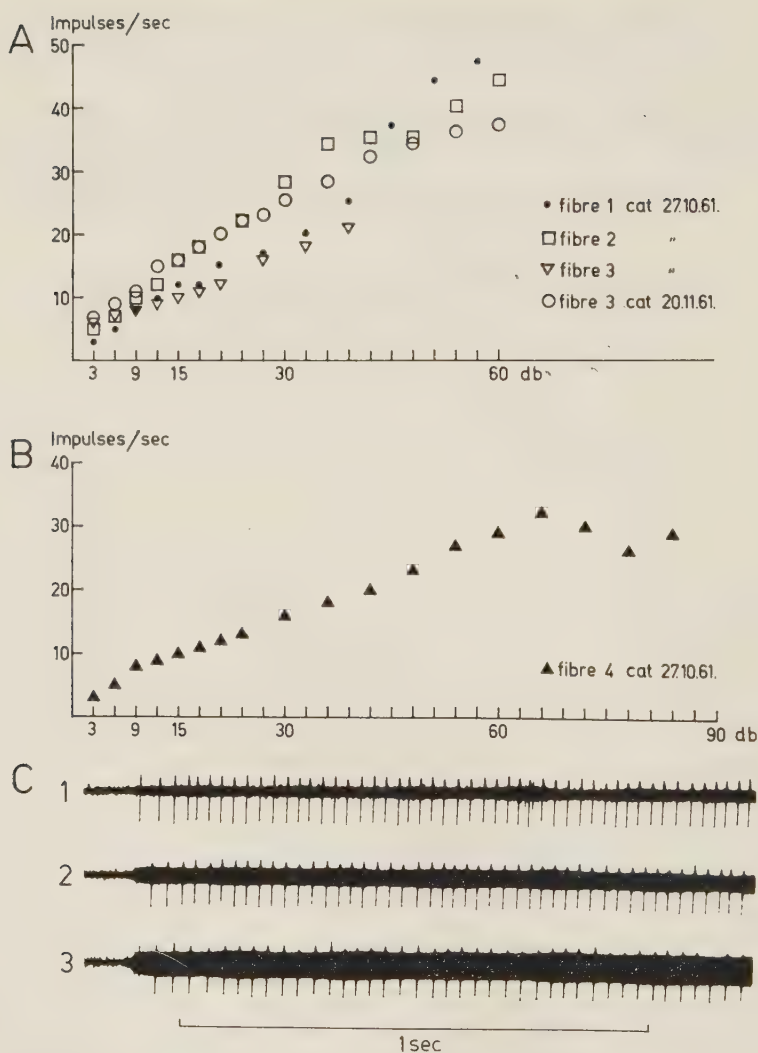


Fig. 4. Relation between sound pressure and frequency. In A and B the number of impulses fired during the first second of the presentation of a long sound pip has been plotted against sound pressure relative to the threshold of each of the fibres tested. The four fibres represented in A never reached a plateau. The fibre in B, however, reached a maximal firing rate 66 db above its threshold. The responses of this fibre to pips 66, 72 and 78 db above threshold are shown in C. Note that the latency in the records of C increases with increasing sound pressure. The beginning of the sound stimulation corresponds to a thickening of the base line. Note also that the first interspike interval in C1 is longer than any one else in C1. Note also the regular firing rate.

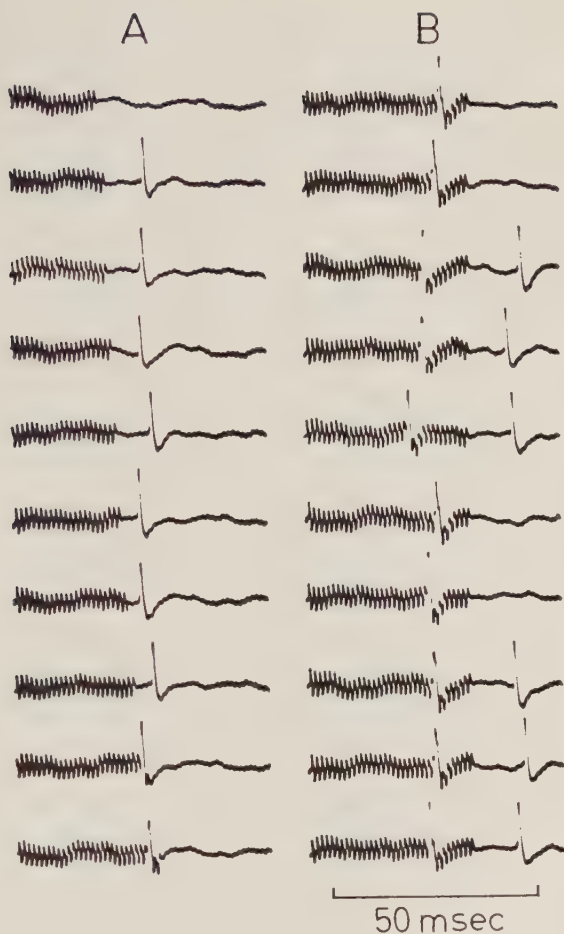


Fig. 5. Responses of a crossed fibre to loud tone pips of increasing duration. Note that the shortest tone pip in A did not evoke an impulse. In B where tone pips are longer than in A, a second impulse appears. Tone frequency of tone pip 820 c/s. Cat 26.07.61.

Threshold determination

When a fibre had been identified as a crossed olivo-cochlear fibre the attenuator was set so as to give a moderately strong output of sound and the sine wave generator's frequency bands of 200—20 000 c/s were scanned. If the fibre was activated by sound the frequency band giving the highest impulse frequency was scanned again, this time at a much lower sound pressure level than the first time. By stepwise production of sound pressure changes it was possible quickly to find the lowest sound pressure level within ± 3 db at which the fibre could be activated. This "lowest sound pressure level" will be called the fibre's threshold and the sine frequency, at which the threshold was found, will be referred to as the fibre's "best frequency". The transients produced

when the connection between the tone generator and attenuator was plugged or unplugged did not confuse the issue. The loudspeaker system was not corrected for deviations from an ideally flat frequency response curve.

Often sound pips beyond a critical length were needed for stimulation of a fibre, as in Fig. 5. This figure illustrates a response to loud tone pips of increasing duration given at the rate of one per second. Pips of 23 msec duration and more activated the fibre, while pips of 21 msec duration did not, as seen in A. In B a second spike appeared in association with a longer pip. This indicates that temporal summation may be essential for the triggering of the crossed olivo-cochlear neurones. It cannot be decided here, however, whether this takes place at the level of the crossed olivo-cochlear neurones or at an earlier stage.

Every unit tested in the way described had a well defined threshold within ± 3 db, provided that sound pressures markedly above threshold level had not been used for long durations. Strong sounds often increased the threshold. This may be exemplified by unit 2 in cat 27.10.61. The threshold was found at the attenuator setting -57 db. Sound pips were then presented with stepwise increase of sound pressure up to 0 db attenuation. The pips were about $1\frac{1}{2}$ sec long, and the intervals between them were of the same order. Some 20 sec after the last pip the threshold setting was -48 db. Pips up to maximal output were again given and 10—15 sec later the threshold setting was -42 db. The basis for the rise of threshold could be inherent in the crossed olivo-cochlear nerve cell or it could be secondary to events in primary and secondary auditory neurones. The following observation favours the latter alternative. ROSE et al. (1959), working with single units of the cochlear nuclei, found recovery times of minutes after moderate tones of short duration.

Although absolute sound pressure levels were not always recorded it can safely be said that most of the units activated by sound did not respond below 40 db SPL and many of them not below 60—70 db SPL. On the other hand, a few units responded at very low sound pressure levels. For two such units the sound pressures at the tympanic membrane were measured with a condenser microphone; for a third, the sound pressure was extrapolated from such a measurement made previously. Thus unit 2, cat 10.11.61, was activated by a 4 000 c/s tone at $15 \text{ db} \pm 3 \text{ db SPL}$; unit 12, cat 14.11.61, by a 5 500 c/s tone at approximately 15 db SPL and unit 3, cat 20.11.61, by a 4 000 c/s tone at 25 db SPL.

Best frequency and band width

Sometimes it could be decided in which fascicles of the vestibulo-cochlear anastomosis the microelectrode tip had been placed (see above, p. 14 and p.

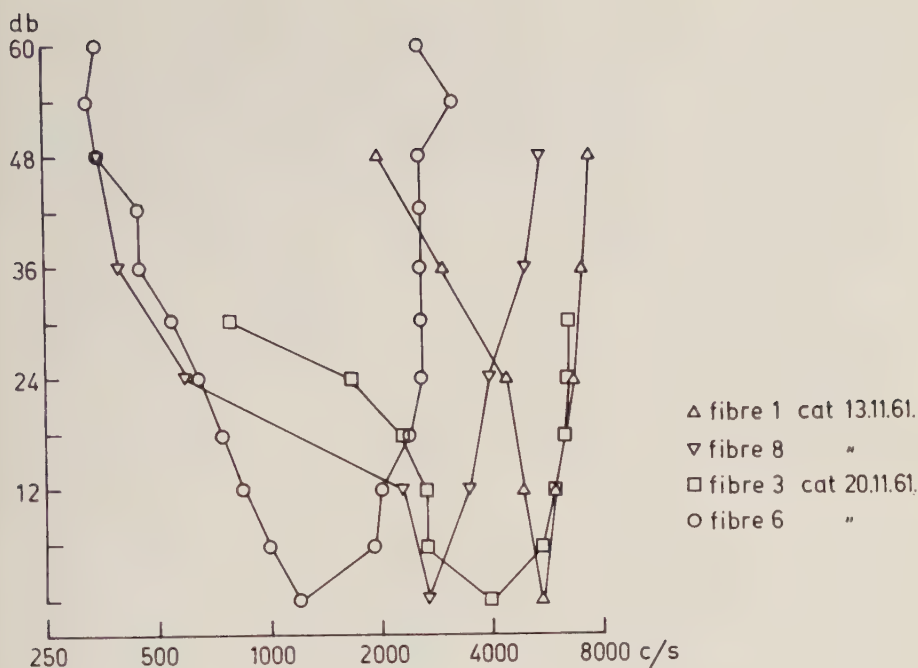


Fig. 6. Diagrams have been plotted for four crossed efferents showing the lowest and highest tone frequencies at which these fibres responded when tested with stepwise increasing sound pressures, relative to the thresholds of the neurones. The absolute values of the thresholds are not given. Note! the cut off at the high frequency side.

15). This made it possible to try to obtain information as to whether or not fibres running to the different parts of the cochlea responded preferentially to different frequencies.

Most crossed olivo-cochlear fibres tested in the way described above had a best frequency (p. 21) determinable within 10 %. Table 1 offers data on all fibres which ran in the most basal or the most apical fascicle of the vestibulo-cochlear anastomosis, as verified at section, and for which best frequencies had been determined. There seemed to be a greater proportion of high best frequencies from the basal than from the apical fascicle. The material, however, is too limited for this to be taken for more than an impression. The highest and the lowest frequencies of sound stimuli activating each fibre was determined for different sound pressures. The results in four fibres have been plotted as sound response curves in Fig. 6, giving a graphical expression of the frequency range at different sound pressures. The threshold at the best

Table 1

cat	fibre	Fibres from the most apical fascicle			Fibres from the most basal fascicle		
		best frequency	db increase	lowest and highest frequency	best frequency	db increase	lowest and highest frequency
04.08.61	1	500					
	2	200					
	3				6 900		
19.08.61	1				2 800—3 000	30	800, 10 000
	2				2 700		—, 10 000
18.09.61	4	1 100					
20.09.61	1—3	2 500					
27.09.61	1	200					
12.10.61	4	2 500—3 000	60	500, 3 500			
25.10.61	1				1 200		—, 10 000
	2				1 100		—, 10 000
	3	1 100					
	4				3 000	30	700, 6 000
	5	900		200, 2 000			
	6	3 000		500, 3 300			
	7	1 300					
31.10.61	1	1 200	24	—, 2 000			
06.11.61	1	5—600					
	4	1 000					
	6	2 000					
10.11.61	2				4 000	18	2 000, 4 600
	4	1 500					
	6	2 000					
13.11.61	1	5 500		—, 7 500			
14.11.61	1	3 500	12	350, 5 550			
	2				6 000		
	3				400		
	4	3 000					
	6	2 400					
	7	1 500					
	2	3 000	18	700, 4 000			
16.11.61	7	3 500					
	10	900					
20.11.61	3	4 000	30	800, 6 500			
21.11.61	1	3 000					
23.11.61b	1	6 000					

frequency determined the coordinates for the minimum of a curve. Since inhibition at intensities high above thresholds has been reported to occur in neurones in the cochlear nuclei (ROSE et al. 1959), the possibility should be mentioned that there may be several threshold frequencies for each frequency level. The curves in Fig. 6 represent at the chosen levels of sound pressure the lowest and highest values within which all possible frequency ranges must lie.

Long tone pips were used as stimuli in these determinations. No correction has been made for the loudspeaker system's deviation from an ideally flat response curve (see above, p. 12).

The sound response curves of Fig. 6 all show a cut off, or a tendency to a cut off, at the high frequency side and the fibres are similar in this respect to primary auditory neurones (TASAKI 1954; KATSUKI et al. 1958) and to secondary auditory neurones (GALAMBOS and DAVIS 1943; TASAKI and DAVIS 1955; KATSUKI et al. 1958; ROSE et al. 1959). Often it was possible to determine the tone frequency range for a fibre at one sound pressure level only. Data from such experiments are given in Table 1. The sound pressures applied corresponded as a rule to the maximal output of the loudspeaker system and could sometimes be expressed in db relative to the fibre thresholds. Of the 9 identified olivo-cochlear fibres in basal fascicles, 6 were tested with high sound frequencies and 4 could be stimulated at 10 000 c/s while none out of 8 tested fibres in apical fascicles could be stimulated at such a high frequency. Looking at the figures for cat 25.10.61 one finds that although there is overlapping of the best frequencies of the apical and basal fascicles, the 3 fibres in the basal fascicle responded to much higher frequencies than did the two apical fibres.

GALAMBOS, SCHWARTZKOPFF and RUPERT (1959) claim that superior olive units respond to a limited region of the auditory spectrum "and their response areas show with rare exceptions the triangular shape and steep drop-off at the high frequency border seen at the cochlear nucleus". This generalization does not seem to hold for the single units of the present study. Of the 6 units mentioned above from the most basal fascicle, 2 responded to a sine wave frequency 3—4 times the best frequency and one to a sine wave frequency twice the best frequency. Such wide band widths relative to the best frequency were also found in some fibres in the middle fascicles of the vestibulo-cochlear anastomosis, while the fibres in the most apical fascicle showed more of a high frequency cut-off.

These findings indicate that crossed olivo-cochlear fibres in the most basal fascicle in the vestibulo-cochlear anastomosis can respond to higher frequencies than can the fibres in the most apical fascicle. They also suggest that fibres in the most basal fascicle can respond over a wider frequency range, expressed as multiples of the best frequency, than do fibres in the apical fascicle.

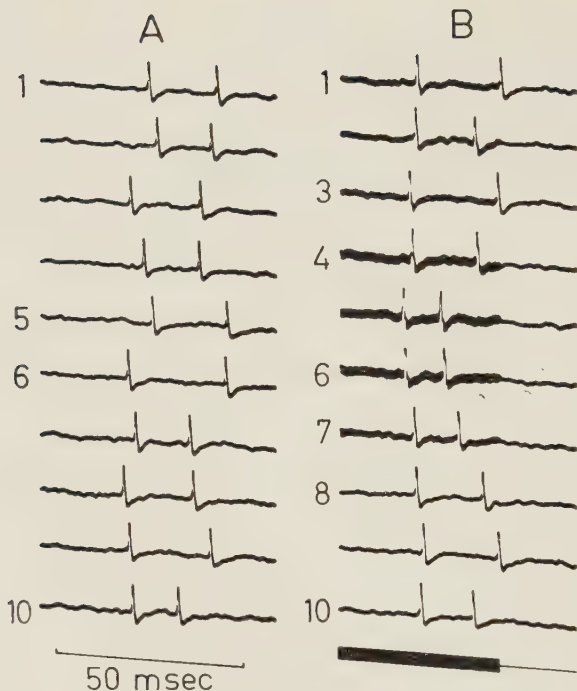


Fig. 7. Responses of a crossed efferent to tone pips. Tone pips were increased in strength between sweeps 5 and 6 in A, between sweeps 10 in A and 1 in B and between sweeps 3 and 4 in B. Note that the latency is decreased with increase of sound pressure. Note also decrease of interspike interval with increase in sound pressure. Sound pressure was again lowered between sweeps 6 and 7 and between sweeps 7 and 8 in B. This neurone was not activated by clicks. Tone frequency of pips 2 250 c/s, pips were given at a rate of 4/sec. The marking below B 10 indicates duration of pip. Cat 19.07.61.

Latency of response to sound

For the latency determinations, short tone pips of approximately best frequencies with rising times of 1—3 msec were presented. The latency of evoked impulses to sound stimuli was defined as the time from the beginning of the sweep to the peak of the impulse.

Latencies between 5 and 40 msec were found in 25 fibres stimulated with tone pips, only 5 of them had latencies below 10 msec. Most units with longer latencies could not be stimulated with very short sound stimuli but behaved like the unit of Fig. 5.

Latency was found to decrease in several fibres with increase of sound pressure, as shown in Fig. 7. Between the 5th and 6th sweep in Fig. 7 A, the sound pressure of the pip was raised, the corresponding latencies decreased



Fig. 8. Responses of a crossed efferent fibre to tone pips. Note that the latency decreased by a step when sound pressure was increased between A and B. Pips were presented at the rate of one pip per second. Cat 18.06.61.

from 19–25 msec to 18–21 msec. In Fig. 7 B the sound pressure was increased twice. Sweeps 1–3 and 4–6, with latencies of 15–16 and 13.5–15.0 msec respectively, show the effects of the two increases in sound pressure.

In some fibres a stepwise change of latency was found, indicating that there are preferred intervals for firing an impulse. In Fig. 8 the intensity of the stimulating sound pip was greater in B than in A with a resulting latency shift of more than 15 msec. It appeared as if this shift was due to the occurrence of a new spike coming up earlier, the position of original spike remaining unchanged.

Contrary to the tendency illustrated in Figs. 7 and 8 a prolonged latency was sometimes found when fibres were stimulated repeatedly at sound pressure levels at the upper end of their dynamic range. The first spike interval then tended to be longer than the next few intervals. These two points are illustrated in Fig. 4 C 1–3.

It may be significant that out of the 6 units of the exceptionally lively cat 28.09.61, 3 units had a latency less than 10 msec.

Response to electrical shocks to the homolateral cochlear nerve

It has thus been shown above that crossed olivo-cochlear fibres can be activated by acoustic stimulation of the contralateral ear. It would be pertinent for the question concerning an auditory activation of these fibres to investigate whether or not they could be activated also from the ipsilateral side. Natural stimuli could not be used since the auditory function of the ipsilateral inner ear had been destroyed by the dissection. The problem was therefore approached by electrical stimulation of the ipsilateral cochlear nerve.

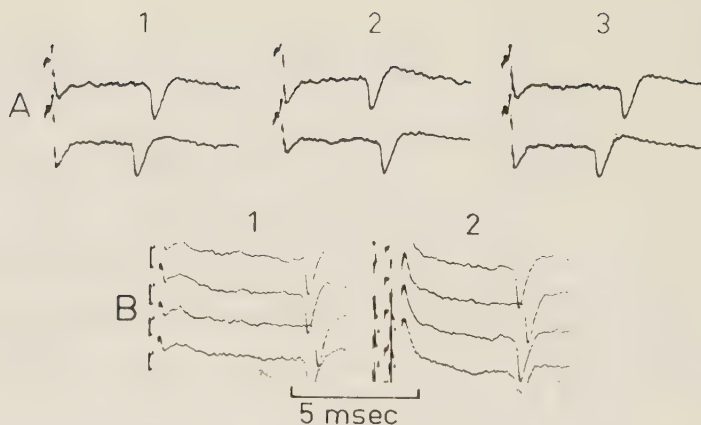


Fig. 9. Responses of crossed efferent fibres in the vestibulo-cochlear anastomosis to electrical stimulation of the ipsilateral cochlear nerve. In A single shocks of two different strengths were given. The size of the shock artifact seen to the left of the sweep indicates the relative stimulus strength in each of the pairs of sweeps in A 1, A 2, and A 3. The larger shock gives the shorter latency. In B the effect of single (1) and paired (2) shocks are compared. The latency is shorter in B 2 than in B 1. A and B are from the same experiment. Cat 28.09.61.

In 20 of the 47 animals, the cochlear nerve was stimulated electrically. The procedures used were the same as described above (p. 15) with the addition that stimulating electrodes were placed in the part of the cochlear nerve peripheral to the basal turn. Single shocks and tetanic bursts at the rate of 1 500 shocks/sec were employed.

The direct response to a single shock was never more than one impulse, and such an impulse had a latency in the range of 3.5—10.0 msec as measured from the beginning of the shock to the peak of the impulse. The latency



Fig. 10. Responses of a crossed efferent fibre in the vestibulo-cochlear anastomosis to electrical stimulation of the ipsilateral cochlear nerve showing regular latencies. Shock rate 4 shocks per second. Cat 22.10.61.

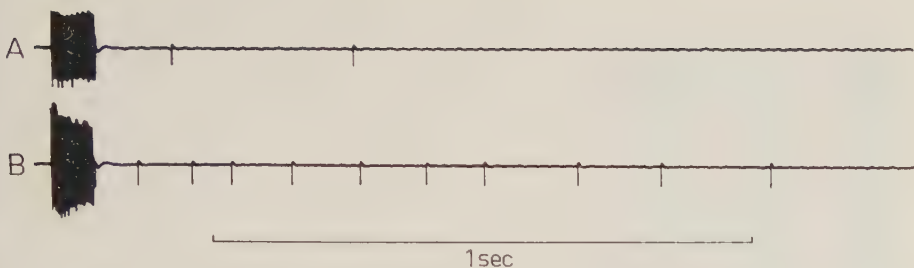


Fig. 11. After discharges in a crossed efferent fibre in response to tetanic stimulation of the ipsilateral cochlear nerve with shock rate 250/sec. Between A and B the shock amplitude was increased from 2 V to 8 V. Other stimulus parameters were unchanged. Cat 16.11.61.

depended somewhat on the stimulus strength used as seen in Fig. 9 A where increased shock strength decreased latencies from 4.8—5.1 msec to 4.0—4.2 msec. In Fig. 9 B a change of stimulus from one shock to two shocks changed the latencies from 6.5—7.0 msec to 6.0—6.3 msec. Latencies to shocks of constant strength well above threshold were typically regular as shown in Fig. 10. Sometimes an impulse appeared with a latency less than 1.5 msec that probably reflected current spread from the stimulating electrode to the olivo-cochlear fibre under study.

In a few fibres repetitive stimulation to the cochlear nerve evoked not only direct responses but also after-discharges which generally increased in rate and duration with increased stimulus strength and repetition rate. The effects of increased stimulus strength on the after-discharge are seen in Figs. 11 A and B.

In two fibres single shocks evoked a direct response only after the fibres had

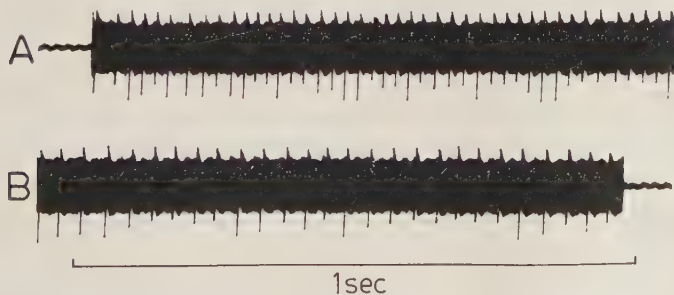


Fig. 12. Beginning (A) and end (B) of a response of a crossed efferent in the vestibulo-cochlear anastomosis to tetanic stimulation during 10 sec of the ipsilateral cochlear nerve. Record of 8 sec between A and B has been omitted. The first interspike interval is longer than the few next intervals. There is no initial burst. The firing rate is regular but has declined from A to B. This neurone could not be activated by sound. Cat 22.10.61. under Thiogenal anaesthesia.

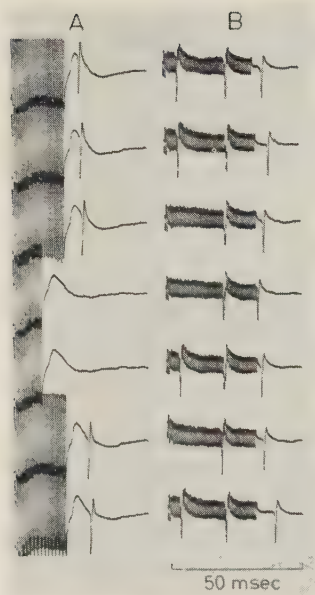


Fig. 13. Responses of two crossed efferents in the vestibulo-cochlear anastomosis to tetanic stimulation of the ipsilateral cochlear nerve. The short bursts in A did not activate the neurone. The longer bursts evoked an impulse. In the record B from another experiment, the impulses appeared with preferred intervals. Note! the long latencies in both A and B. Cat 28.09.61. (A). Cat 10.10.61 (B).

been conditioned with tetanic bursts, an indication of post tetanic potentiation. Fibres that showed post-tetanic potentiation also showed prolonged after-discharges with slow firing rates as in Fig. 11 B.

Repetitive antidromic stimulation with 1—3 shocks/sec, given in the floor of the fourth ventricle, increased the responsiveness of two fibres to single shock stimulation of the cochlear nerve, applied 0.3—1.0 sec after the series of conditioning shocks.

The responses to tetanic stimulation of the ipsilateral cochlear nerve showed some of the characteristics of the effects of sound stimulation, described above, in that there was no initial burst and there was regular firing with low maximal rate as compared with primary and secondary auditory neurones. Fig. 12 illustrates these features as well as the fact that the first impulse interval was longer than the next few and that the firing rate was 41 impulses during the first second and 25 impulses during the last second of the 10 sec long tetanic burst.

Sometimes with tetanic stimulation of the cochlear nerve long latencies were found that were of the same order as those described above for sound stimulation. Fig. 13 A shows how tetanic bursts of 20 msec duration evoked single spikes with latencies of 26—30 msec, while tetanic bursts of 11 msec were ineffective. In B, impulses appeared with preferred intervals and occasionally an impulse coming with a latency of 25 msec had no preceding impulse.

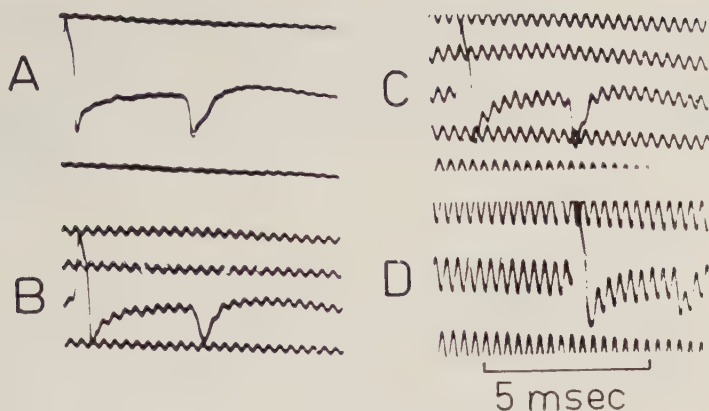


Fig. 14. Sound conditioned responses of a crossed efferent in the vestibulo-cochlear anastomosis to single shocks applied to the ipsilateral cochlear nerve. The conditioning sound was increased between each of the records A—D. This neurone did not respond to unconditioned shocks. Note! the latency of the test response decreased with increase in sound pressure. Tone frequency 3 000 c/s. Cat 21.11.61.

Response to bilateral cochlear nerve stimulation

Out of 32 fibres activated by either mode of stimulation, 20 fibres were activated by both acoustic stimulation of the contralateral ear and electrical stimulation of the ipsilateral cochlear nerve. A convergence of impulses from both cochlear nerves towards crossed olivo-cochlear neurones was thus demonstrated.

To investigate whether signs of interaction between auditory inflow of the two sides could be found in crossed olivo-cochlear activity, the results of simultaneous stimulation of both cochlear nerves was studied in 4 cats (20.11.61, 21.11.61, 23.11.61a, and 23.11.61b).

It was found that acoustic stimulation increased the responsiveness to electrical stimulation in fibres that could not be activated by unconditioned single shocks. Single shocks applied to the cochlear nerve were followed by single impulses in crossed olivo-cochlear fibres with latencies of 3.5—5.1 msec when the shocks were given during presentation of long tone pips. In some fibres the acoustic stimulation was of subthreshold strength; one fibre could not be activated acoustically. In one unit, which could be activated by either of the two modes of stimulation, subthreshold shocks given during sound stimulation were followed by impulses with a latency of 4 msec.

In Fig. 14 is shown how an increase in sound shortens the impulse latency relative to single shocks. This fibre could not be activated by unconditioned single

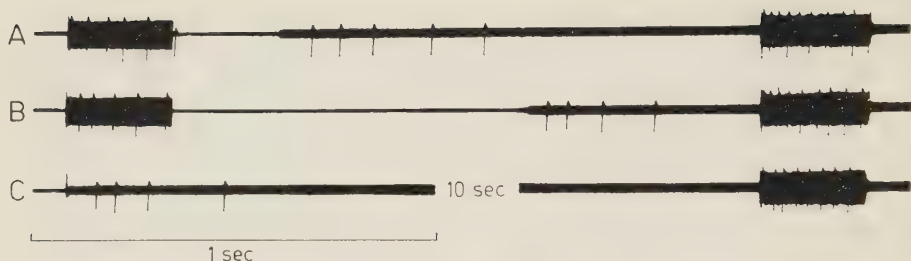


Fig. 15. This figure shows the response of one crossed efferent in the vestibulo-cochlear anastomosis to tetanic stimulation of the ipsilateral cochlear nerve. To the left in A and B are shown the responses to unconditioned tetanic bursts, to the right in A, B and C are shown the responses to tetanic bursts conditioned by sound. Note! the increased number of impulses when the tetanic bursts were sound conditioned. No tetanic burst was given in C before the one illustrated. Note that only 4 to 5 impulses were evoked by the tone at its beginning. The frequency of the conditioning sound was 2 500 c/s. Cat 21.11.61.

shocks. The increased responsiveness of a fibre to bilateral cochlear nerve stimulation is illustrated in Fig. 15. As shown in A and B of this figure 6 nerve impulses were evoked by a tetanic burst without a background of sound stimulation and 9—10 impulses were fired when the burst was given concomitant with sound. Fig. 15 C illustrates that the facilitating effect of sound remained after 10 sec of sound stimulation; the fibre fired a few impulses only at the beginning of the pip. There was also a change of latency of the first impulse in the burst, from 5.1 msec without the tone to 4.6 msec when the tone was presented.

Increase of responsiveness was demonstrated in 11 out of 12 fibres. In the one exception the fibre could be activated by sound but not by electrical stimulation alone and single shocks or tetanic bursts of shocks applied to the cochlear nerve were not followed by impulses in any constant time relationship when sound was presented together with the shocks.

CHAPTER II

EFFERENT INFLUENCE ON SINGLE PRIMARY AFFERENTS

In this section of the work effects of electrical stimulation of the crossed olivo-cochlear fibres on the activity of the primary auditory fibres have been studied. Since RASMUSSEN has described crossed olivo-cochlear connections with the cochlear nucleus as well as with the cochlea proper, it was considered necessary to have a valid criterion for differentiating primary from secondary auditory neurones.

The value of a criterion based on short latencies to sound seems questionable in view of the results of KATSUKI, SUMI, UCHIYAMA and WATANABE (1958) who found latencies ranging from 1—5 msec in the dorsal cochlear nucleus of cats. TASAKI (1954) reported time intervals from 1.1—1.3 msec between the start of the microphonic response and the earliest single primary auditory fibre response to strong 8 000 c/s pips (in guinea pig). In the absence of a satisfactory electrophysiological basis for identifying primary auditory neurones, a histological criterion was established; the following studies were pertinent in this regard.

ALEXANDER and OBERSTEINER (1908) described findings of glial islands in the cochlear branch of the eighth nerve in man, peripheral to the glial septum. In longitudinal sections of the eighth nerve stained by the Nissl method, a light, central region can be differentiated from a dark, peripheral region. The transition zone is concave centrally and represents the glial septum (ALEXANDER and OBERSTEINER 1908). Somata of secondary auditory neurones are found central to this septum; the small grain cells are located close to the septum while the large, multipolar nerve cells are more centrally placed (see RAMON y CAJAL 1952).

TARLOV (1937) confirmed the findings of ALEXANDER and OBERSTEINER in man and stated that "confirmatory observations were made on nerves from monkeys, cats and rabbits — — —".

GALAMBOS and DAVIS (1948) described the presence of nerve cell bodies in histological sections of the intracranial part of the eighth nerve of cats.

CONTU (1958) referred to a work by CONTU, NEVES PINTO and SANTOS (1956) in which by using a silver method based on work by RAMON y CAJAL and De CASTRO the authors found large multipolar nerve cells in the cochlear

division of the eighth nerve in cat. It was not stated how far peripherally the cells were found or of what size they were. They were believed to be secondary auditory nerve cells.

In the present work the cochlear division of the eighth nerve was investigated histologically. The findings—to be described—led to the conclusion that no secondary auditory neurones occur peripheral of the main glial septum in the nerves studied. Accordingly (see below) criteria were developed for locating the recording microelectrode to this site.

Technique and Procedure

Selection of experimental animals

The experiments in this series were carried out on 12 cats weighing between 1.8 and 3.2 kg. They were chosen by the criteria described above (see p. 8). Animals in which blood collected in the cochlea during manipulations of the eighth nerve were discarded. In order to determine the presence of blood in the cochlea it was found practical to have the bulla open during the experiment to permit inspection of the inner ear through the round window membrane.

The preparation of the animal for the experiment

The animals were decerebrated, stimulating electrodes were placed in the floor of the fourth ventricle and the bulla was exposed as described above (p. 9). In cat 08.12.61 a deep incision was made to cut the olivo-cochlear bundles 2 mm to the right of the stimulating electrodes in the floor of the fourth ventricle. In 6 animals round window electrodes were used for monitoring inner ear activity and in 2 cats the middle ear muscles were divided.

The region of the left cochlear nuclei was freed from the overlying part of cerebellum. The final removal of cerebellar substance by suction at the internal auditory meatus was done under the microscope with glass tubes drawn to tip diameters of 0.5—1.0 mm and flamed. The "Heparin flushing technique" (p. 10) proved to be of importance here too for a successful preparation. Of even greater importance was the use of a 30 % urea solution with 10 % inert sugar to prevent edema of the brainstem (see p. 14). Such edema always changed the topographical relationships between the internal auditory meatus and intracranial part of the eighth nerve making the successful approach with a microelectrode to the eighth nerve difficult or impossible. The third crucial technical device for this work was to anchor the intracranial part of the eighth nerve to the bone with insect needles (see above, p. 13), which gave relatively stable recording conditions. Gel-foam was used to stop bleedings in this part of the preparation, and sometimes for closing an opened petrous sinus.

Stimulating technique

The stimulator and stimulating electrodes were described above (p. 10) as were also the acoustical equipment and the noise level of the room (p. 12).

Recording technique

Capillary microelectrodes filled with 4 M NaCl or with 3 M KCl and having a resistance, as measured in the preparation, of 10—40 megohms were used for recording from single auditory neurones. The activity was monitored and recorded as described above (p. 12). Chlorided silver-wires were used to record cochlear signals from the round window. They were connected to an AC-coupled differential amplifier feeding the second beam of the split beam cathode ray tube.

Control of the topography and histology of the cochlear division of the eighth nerve relative to the position of the micropipette tip

For recording the micropipette was inserted into the intracranial part of the eighth nerve close to the upper margin of the internal auditory meatus. It was then advanced towards the apex of the modiolus at a direction almost tangential to the eighth nerve. Recording was started when the electrode tip was considered to be peripheral to secondary auditory neurones. Branchings of surface vessels were used as references for localizing the point where the electrode tip had entered the intracranial part of the eighth nerve.

The length of tissue transversed by the electrode tip was approximated on the scale of the micromanipulator. Such an approximation is defended by the following argument. Had the microelectrode pushed with it the nerve the action potentials recorded would probably have changed as if the electrode tip had slowly been pushed further. Such changes of the action potentials were not the rule.

When the experiment was finished, the micropipette was withdrawn and its insertion point was marked with a finely ground tip of platinum wire dipped in Indian ink. The holder and manipulator were left in position, thus maintaining the direction of the micropipette. The animal was then killed with Thiogenal. The eighth nerve was dissected with care being taken not to change its topographical relationship to the remaining bone structures during removal of tissues over the lateral side of the nerve. Following the dissection the tip of the micropipette was lowered again and the binocular operating microscope was placed so that a right angle was formed between the optical axis of the microscope and the long axis of the micropipette. With the microscope in this position and with the aid of a measuring ocular distances along the eighth nerve were measured as if they had been projected on the pathway of the

microelectrode tip. The eighth nerve was marked with Indian ink at a given distance of 2 or 2.5 mm from the first ink spot. A piece of cotton soaked in a 10 % formalin solution was then placed upon the eighth nerve and left there for about one hour. Then one transection was made at right angles to the direction of the micropipette central to the Indian ink spot in the intracranial part of the eighth nerve and another transection was made peripheral to the second ink spot. The cochlear division of the eighth nerve together with the cochlear nuclear region between the two transections was removed for fixation in 10 % formalin. Histological sections, 12 μ thick, were cut parallel to the transection in the cochlear nuclear region, and stained for Nissl substance.

The two ink spots were easily identified in the stained sections of the cochlear nerve. Preliminary experiments demonstrated that shrinkage of the preparation was uniform. The thickness of a histological section could thus be expressed in terms of distance travelled by the tip of the micropipette. The distance was known from the settings of the micromanipulator and in this way the recording sites of the microelectrode could be determined approximately. Edema was the main source of error since it not only made the choice of site for insertion of the pipette tip difficult but sometimes also made it impossible to identify this site at the end of the experiment. Blood clots could have the same effect. With the introduction of urea to prevent edema (see above) and Heparin locally to prevent blood clotting (see above) these difficulties were removed from most experiments. Still it was considered safe to allow for a wide margin of error and many of the observations reported were done with the microelectrode tip advanced more than 1.5 times the distance that seemed sufficient to satisfy the criterion for recording from primary auditory neurones (see below, p. 37).

It should be stressed that the procedures described above precluded recording from afferents from the most basal part of the cochlea. The primary neurones from the basal part of the cochlea can be reached from the internal auditory meatus with a straight micropipette, but the relatively short distance from the region of the secondary auditory neurones to the bony cochlea gives a narrow margin of safety. A technique with steel microelectrodes permitting exact marking (GREEN 1958; modification suggested by GREEN in BROWN and TASAKI 1961) would be advisable in work with primary auditory neurones from the basal turn, if recording from these neurones is feasible with such electrodes.

Conduction of the experiment

The tip of the micropipette was placed in the region of the cochlear nuclei under microscopic control. Fine glass tubes with tip diameters of 0.2–0.5 mm were used for sucking away the cerebro-spinal fluid from the dorsal surface

of the intracranial part of the eighth nerve at the internal auditory meatus. The input cathode follower was temporarily connected to ground over a grid leak of 22 megohm. The change of noise in the headphones marked the contact between the cochlear nuclei region and the tip of the electrode. The setting of the micromanipulator was registered and the resistance of the pipette was measured. The pipette was advanced about 2.5 mm in one minute and if the pipette resistance did not change the actual recording began. From now on the animal was curarized with Flaxedil, 6—10 mg/kg body weight.

Results

Histological features pertinent to recording from primary auditory neurones

With respect to the possibility of recording from primary auditory neurones as differentiated from secondary neurones (see above, p. 33) by the technique described above (p. 35), the histological findings were promising. Multipolar nerve cells, of the variety found central to the main glial septum, were never seen peripheral of this septum in the 36 auditory nerves studied. The transition from the central to the peripheral portion of the cochlear branch of the eighth nerve is sometimes irregular, presenting gaps which allow tongues of glial tissue to project peripherally, findings which confirm those of ALEXANDER and OBERSTEINER (1908) and of TARLOV (1937).

The distance from the insertion site of the microelectrode in the intracranial part of the eighth nerve to the most peripheral part of the main glial septum was found to vary between less than 1 mm to more than 3.5 mm. The main part of this variation was in the length of the central part of the cochlear division of the eighth nerve, as measured from the somewhat poorly defined upper margin of the internal meatus. Because of this variation each eighth nerve recorded from was studied histologically.

Criterion

In this study nerve impulses activated by sound have been recognized as coming from a primary auditory neurone only if the recording site of the microelectrode was localized to the part of the cochlear division of the eighth nerve that was peripheral to the main glial septum. By this criterion, however, the efferents were not excluded.

Several groups of fibres were found which could not be activated by sound and which had a resting activity that was much more regular than that of auditory fibres. This regular discharge could sometimes be accelerated by tetanic electrical stimulation of the floor of the fourth ventricle, but there was never a one-to-one relationship between shocks and impulses, and no response

to single shocks as monitored by cathode ray tube and the headphones. Inspections after such experiments always revealed that the direction of the micropipette had been inadequate for recording of primary auditory fibres and that the tip of the microelectrode had been in the vestibular portion of the eighth nerve. No systematic analysis of these findings was performed. It was possible that efferent vestibular fibres (RASMUSSEN and GACEK 1958, GACEK 1960) were stimulated giving a change of activity in vestibular afferent fibres.

Effect of electrical stimulation of crossed olivo-cochlear fibres on activity in primary auditory neurones

In this material consisting of 56 primary auditory fibres there was not one instance of facilitation of resting or sound-evoked activity by electrical stimulation administered to the floor of the fourth ventricle. In all but 4 of the 56 fibres inhibition was found when the floor of the fourth ventricle was electrically stimulated. These 4 fibres had no other recorded characteristics by which they could be differentiated from the other 52 fibres. The last two cats in this study gave the most stable recording and stimulating conditions; 9 primary auditory fibres were studied in one and 11 in the other. In each of these two experiments great differences in sensitivity to olivo-cochlear stimulation were observed even between two consecutively studied fibres. However, the interesting question if there are different classes of primary auditory fibres relative to sensitivity to crossed olivo-cochlear inhibition has not been studied.

Resting activity was found in many fibres. In several instances it was not possible to decide whether such activity was spontaneous or in fact caused by the noise in the room (see p. 12). However, when it appeared in fibres with thresholds 20–40 db above the noise level of the room it was concluded that it was true spontaneous activity. This activity could be inhibited by electrical stimulation of the crossed olivo-cochlear fibres if sound evoked activity was inhibited by such stimulation. Inhibition of resting activity is illustrated in Fig. 16. One click a second against a background of resting activity was presented with a delay of about 10.5 msec relative to the signal triggering the sweep. The click response consisted of one impulse. In B a brief tetanus of shocks to the floor of the fourth ventricle was initiated at the beginning of each sweep. The resting activity following the click responses was inhibited, but click evoked impulses and impulses preceding the clicks were still found.

Fig. 17 shows inhibition of click evoked impulses. The click was just strong enough to give at least one impulse. In Fig. 17 A the clicks were presented with a delay of 15.5 msec relative to the signal triggering the sweep and the tetanic burst. The first tetanic burst gave an inhibition, the others did not. In

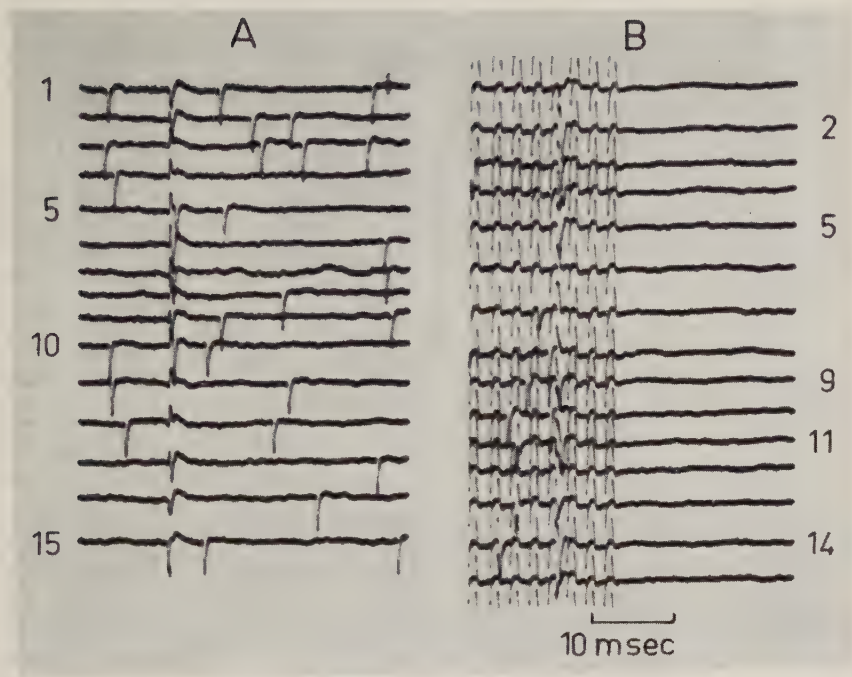


Fig. 16. The effect of tetanic stimulation of crossed efferents on the resting activity in a primary auditory fibre. Clicks presented with a delay of 10.5 msec relative to the beginning of the sweeps triggered one impulse in most of the sweeps in both A and B. In addition tetanic bursts were triggered simultaneously with the sweeps in B. The resting activity before the click is represented by 6 impulses in A and by 5 impulses in B. The number of click evoked impulses in A and B is approximately the same. Note that no impulses are seen in B after the click evoked ones. Decerebrate cat curarized with Flaxedil.

Best frequency of primary auditory fibre 10 000 c/s. Distance from electrode insertion point to most peripheral part of glial septum 1.6 mm, recording from a depth of 2.3 mm. Cat 09.01.61.

Fig. 17 B the clicks were delayed 17.5 msec relative to the start of the burst and under these conditions three out of four tetanic bursts did inhibit the click response. Tetani initiated less than 15 msec before the click failed to inhibit its response. The minimal duration of stimulation required for inhibition in this and other fibres was of the same order when the same stimulus frequency was used (425 shocks/sec).

That considerable time is needed for building up full inhibition of the activity in a primary auditory neurone is illustrated in Fig. 18. Trains of shocks at the frequency of 250 shocks/sec were triggered with sweeps. The sweeps

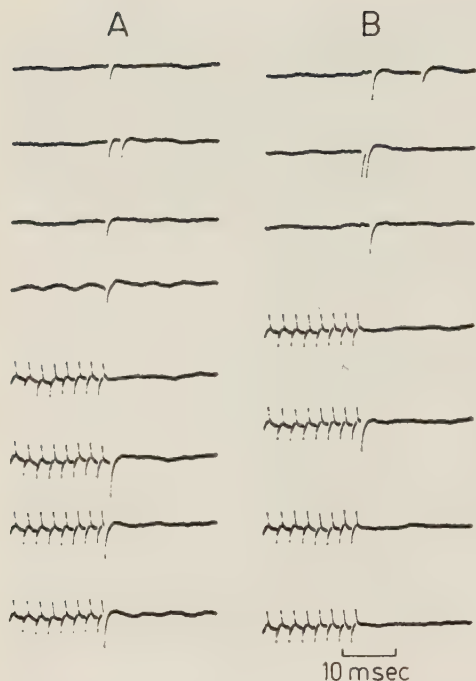


Fig. 17. Effect of tetanic stimulation of crossed efferents on click evoked activity in a primary auditory fibre. The click was chosen just strong enough to give at least one impulse. The tetanic burst was triggered simultaneously with the sweep; the click was delayed less in A than in B. Note that the first tetanic burst in A inhibited the click response. Note that in B the click response is inhibited 3 times out of 4.

Best frequency of the primary afferent was 10 000 c/s. Distance from electrode insertion point to most peripheral part of glial septum 1.6 mm; recording from a depth of 2.3 mm. Cat 09.01.61.

were consecutive and occurred at 2 sec intervals. The auditory fibre was stimulated with a tone 18 db above the fibre threshold. It is seen that the inhibitory effect of tetanic stimulation needed up to 75 msec to be fully developed and inhibition lasted for 70 msec after cessation of the tetanic stimulation.

Another characteristic of the inhibition induced by electrical stimulation of the olivo-cochlear bundle was that it tended to dissipate during prolonged stimulation. With adequate choice of parameters of stimulation, dissipation of inhibition could be found after some tenths of a second. This is illustrated in Fig. 19. The primary auditory fibre was stimulated with sound at more than 5 db but less than 10 db above threshold and then with 5 db increases in sound pressure between each tetanic burst applied at 2.5 to 3.0 sec intervals. After the period of maximal inhibition (Fig. 19 D) nerve impulses began to appear 230—270 msec following the beginning of the tetanic burst.

The degree of inhibition varies with stimulus strength and increases with the shock frequency up to 250 or 425 shocks/sec. Such frequencies were more effective than 150 or 800 shocks/sec. Fig. 18 demonstrates that activity

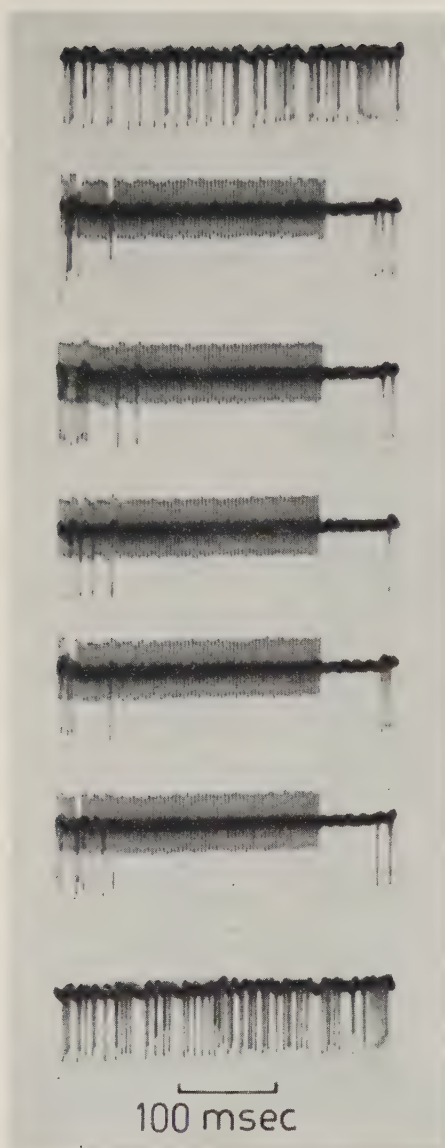


Fig. 18. Effect of tetanic electrical stimulation of crossed efferents on tone evoked activity in a primary auditory neurone. The primary afferent is stimulated with a tone of its best frequency, 10 000 c/s at 18 db relative to its threshold. Tetanic bursts were triggered simultaneously with the sweeps. Note that stimulation of 75 msec was needed to yield full inhibitory effect and that there is a post-stimulatory inhibition of 70 msec after cessation of stimulation.

Same neurone as in fig. 16, same stimulating parameters, except for duration of tetanic burst.

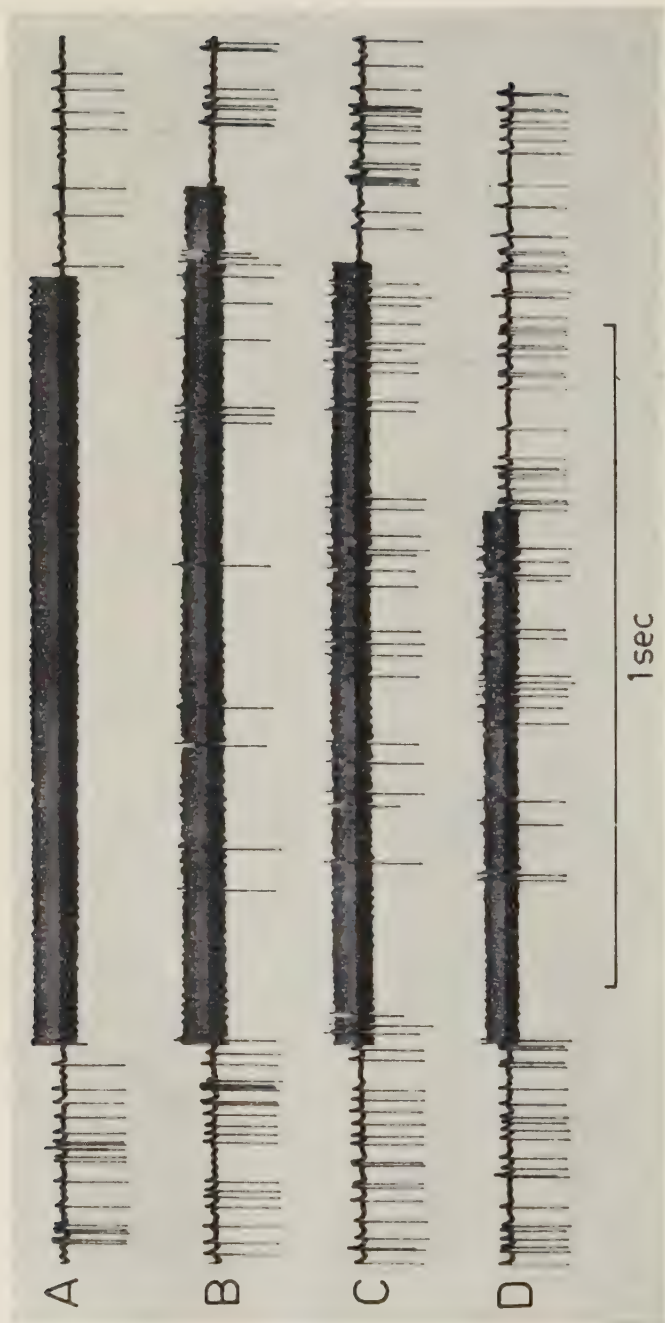


Fig. 19. Effect of tetanic electrical stimulation of crossed efferents on tone evoked activity in a primary auditory neurone. The primary afferent is stimulated with a tone of its best frequency 950 c/s. In A the sound pressure was kept at a constant level between 5 and 10 db relative to the threshold of the fibre. Between each record the sound pressure was increased with 5 db. Tetanic bursts were applied to the efferents. Note the total inhibition in A and some post stimulatory inhibition after cessation of stimulation. Note that in B, C and D there was a dissipation of inhibition from efferent stimulation after about a quarter of a second.

Middle ear muscles cut. Distance from electrode insertion point to most peripheral part of glial septum 1.7 mm; recording from a depth of 2.4 mm. Cat 06.12.61.

evoked by a continuous tone more than 20 db but less than 25 db above the fibre threshold was totally inhibited during 240 msec by electrical stimulation of the crossed olivo-cochlear fibres. In another fibre the activity evoked by a continuous tone 24 db above threshold was abolished for some tenths of a second in response to such stimulation.

Rebound after inhibition was never observed, suggesting that the inhibitory effect is pure.

CHAPTER III

COCHLEAR POTENTIALS CHANGED BY EFFERENT STIMULATION

The results of GALAMBOS (1956) suggest that the crossed olivo-cochlear fibres act somewhere in the region where acoustic energy is transformed into nerve impulses. The result of the many studies concerned with this transmission mechanism (see DAVIS 1961) are used in the present work for a further analysis of the site of action of the system under study. The action of strychnine as reported by DESMEDT and MONACO (1959, 1960) as well as the results to be described here add further information as to how efferent endings may be functionally localized.

Technique and Procedure

Selection of experimental animals

The experiments in this series were carried out on 80 cats weighing between 1.5 and 3.5 kg. They were selected by the criteria described above (p. 8).

The preparation of the animal for the experiment

Most of the animals were decerebrated but some were kept under narcosis with Thiogenal. Stimulating electrodes were placed in the floor of the fourth ventricle and the bulla was exposed as described above. In several animals the middle ear muscles were divided.

Stimulating technique

The technique and the equipment used for electrical as well as for acoustical stimulation have been described above (pp. 10 and 11).

Recording technique

Chlorided silver wires with a diameter of 0.1—0.2 mm were used as electrodes; one electrode was placed on or near the round window and another was located a short distance away as a reference electrode. The wires were enclosed in thin polyethylene tubing and, for stabilization, the tubes and wires were knotted and embedded in dental cement on the rim of the bulla. The bulla was closed with dental cement in most of these animals.

In 3 cats, pipettes filled with a saturated KCl solution and linked through a KCl-agar-bridge to calomel electrodes were used for recording. The tip diameters of the pipettes were of the order of 0.1—0.2 mm. One pipette was placed on the rim of the round window, touching but not stretching the round window membrane. The reference electrode was placed on the medial wall of the bulla. The calomel half-cells (Beckman fibre junction reference electrode No. 39270) were connected to the input cathode followers of a DC-coupled differential amplifier.

The ground electrode was of the same kind as the recording electrodes. In most animals recordings were taken from the left round window only.

Stability of the preparation

During preliminary experiments it was found that changes of the position of the electrode relative to the membrane, too small to be visible under the dissecting microscope, caused gross changes in the size of the recorded cochlear microphonics. The effects of such movements were abolished by drilling a small niche in the rim of the round window: the electrode tip could be moved in this niche without concomitant changes in the size of the cochlear microphonics.

In the experiments reported the animals were curarized and/or the electrode was placed in such a niche in the rim of the round window.

Results

Effects on the action potential component of the round window response

The material presented in a preliminary report (FEX 1959) is included in the present series of experiments on 80 animals.

It was found that electrical stimulation of the olivo-cochlear bundle in the floor of the fourth ventricle reduced or abolished the action potential component of the round window response to a click. The effect was dependent upon the choice of stimulating parameters. The optimal stimulus frequency for giving an effect was found to be of the order of 250—425 shocks/sec. The same frequency range was found to be optimal for the inhibition of primary afferents, as described above (p. 40). With 2 V shocks given at the rate of 250 shocks/sec the inhibitory effect on the action potential needed a few tenths of a second to be fully developed. The same order of time was found for the decay of the effect following stimulation. These time relations have been described by GALAMBOS (1956) and have not been studied in further detail here.

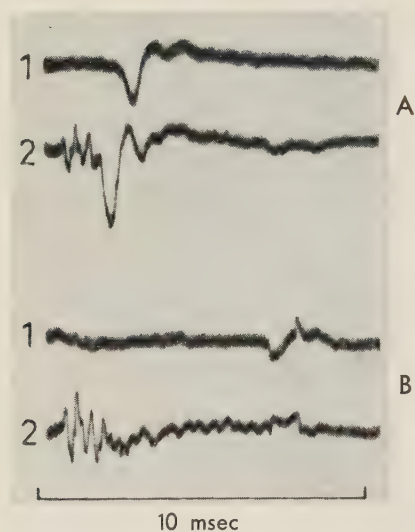


Fig. 20. Effects of electrical tetanic stimulation of the crossed efferent fibres on click evoked responses at the round windows. A shows the control response to a click at the left (1) and the right (2) round window. In B an identical click is presented during prolonged tetanic stimulation. The action potential is abolished in 1 and the cochlear microphonic is increased in 2.

Cat 05.05.59, anaesthetized with Thio-genal and curarized with Flaxedil. Loud-speaker at right ear.

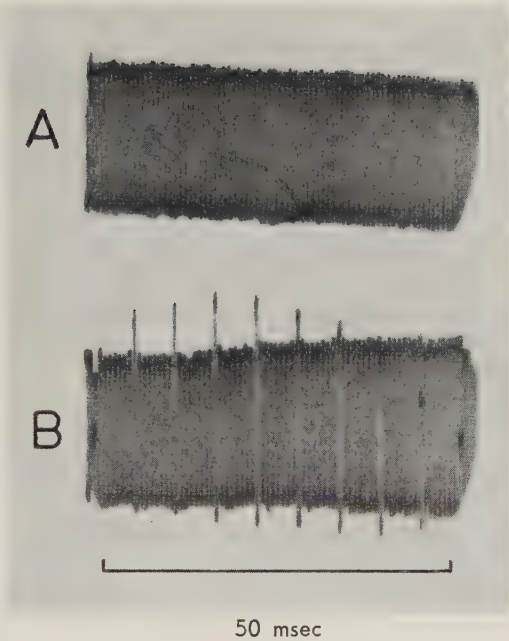
Effects on the cochlear microphonic potential

The cochlear microphonic component of the round window response to sound was increased when tetanic stimulation was applied to the crossed olivo-cochlear bundles. This effect became noticeable at approximately the same stimulus strength that produced a reduction of the action potential component of the round window response. The minimal stimulus strength producing an effect corresponded to a stimulator output of 0.16 V. With increasing stimulus strength the increase of the cochlear microphonic passed a maximum and at a stimulus strength 5—10 times threshold the increase changed to a decrease. Since this change of sign of the effect on the cochlear microphonic was not seen in animals that were curarized with Flaxedil or in animals that had had the middle ear muscles cut, it was considered an effect of current spread to nerve fibres innervating the middle ear muscles.

The increase of the cochlear microphonic with electrical stimulation of the olivo-cochlear bundle was obtained when tested with clicks (Fig. 20) as well as with continuous tones (Fig. 21). It was apparent for tone frequencies from 500 c/s to 9 000 c/s. Tones beyond this range were not investigated. The well-known phenomenon was identified that the cochlear microphonic grows with increasing sound pressure to reach a maximum after which the cochlear microphonic gets smaller with further increase of sound pressure (see e. g. DAVIS 1957). However at sound pressures giving maximal cochlear microphonic, tetanic stimulation of the crossed olivo-cochlear fibres increased the cochlear microphonic still further as it did at sound pressures both below and above this level.

Fig. 21. Effect of tetanic electrical stimulation of the crossed efferent fibres on the cochlear microphonic at the round window. A continuous tone has been presented in A and in B. In B a tetanic burst has been triggered simultaneously with the sweep. Note that there is an increase of the cochlear microphonic with a delay of 17–20 msec relative to the beginning of the burst.

Cat 26.10.59, anaesthetized with Thiogenol and curarized with Flaxedil. Frequency of stimulating tone 1 500 c/s.



The suppression of the action potential at the round window reportedly begins 20–30 msec after the first shock in a tetanic train (GALAMBOS 1956). Approximately the same time relationships were found in the present studies for the increase of the cochlear microphonic. As shown in Fig. 21, the increase

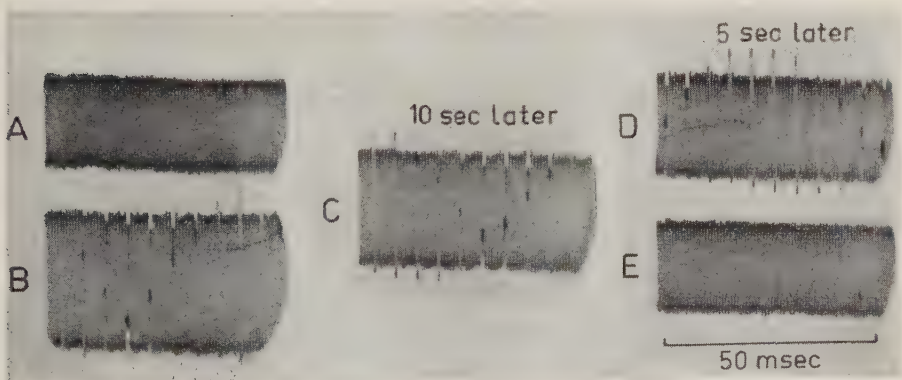


Fig. 22. Effect of tetanic stimulation of the crossed efferent fibres on the cochlear microphonic at the round window. A and E show the control response. In records B–D uninterrupted tetanic stimulation was applied to the floor of the fourth ventricle. Note! the decrease in augmentation of the cochlear microphonic from B–D. Same cat and same stimulation parameters as in fig. 21.

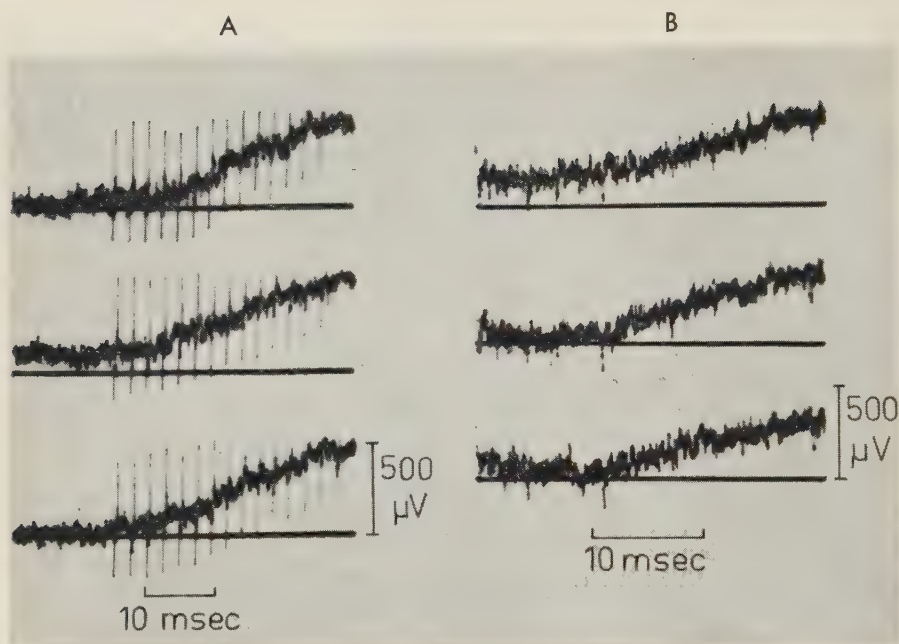


Fig. 23. Effect of tetanic electrical stimulation of the crossed efferent fibres on the resting potential at the round window in two different experiments. In A tetanic bursts were triggered with a delay relative to the sweep, as is evident from the shock artifacts, in B the tetanic bursts and the sweeps were triggered simultaneously (shock artifacts not clearly visible). In A is seen how the resting potential begins to increase after a latency of the order of 7 msec. In B this latency is of the order of 12 msec. Decerebrate cat with middle ear muscles cut, curarized with Flaxedil. Calomel electrodes. Cat 29.05.61. (A). Decerebrate cat, curarized with Flaxedil. Ag—AgCl electrodes. Cat 07.02.61. (B).

of the cochlear microphonic became evident 17—20 msec after the beginning of the tetanic burst.

In many experiments it was noted that the increase in cochlear microphonic tended to diminish with prolonged tetanic stimulation, as shown in Fig. 22. The time relationships of this phenomenon have, however, not been analyzed in this study.

The increase of the cochlear microphonic was maximally of the order of 3 db (Fig. 22 B) and was dependent on the parameters of the electrical stimulation in that it increased with increasing stimulus strength from threshold strength up to levels 5—10 times threshold. The optimal stimulus frequency for effects on the cochlear microphonic, the action potential and the resting potential at the round window (see below, p. 49) was found to be 250—425 shocks/sec.

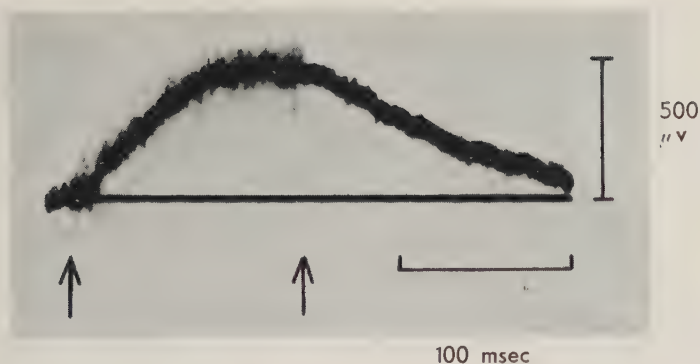


Fig. 24. In this figure is shown the effect of tetanic electrical stimulation of the crossed efferent fibres on the resting potential at the round window. A tetanic burst has been triggered with a short delay relative to the sweep. The arrows mark the beginning and the end of the burst. Note that the increase of the resting potential has reached a plateau within 0.1 sec and that the prestimulatory level has not been reached within 0.15 sec after cessation of stimulation. Same cat as in fig. 23 A, same stimulation parameters.

Effects on the resting potential at the round window

The endocochlear resting potential was discovered and described by von BÉKÉSY (1950, 1952 a) in the guinea pig. He found an endolymphatic resting potential of +80 mV relative to the perilymph of the tympanic scale; von BÉKÉSY also demonstrated that as a reflection of the endolymphatic potential there was a potential of about +2 mV at the round window membrane relative to a reference electrode located near the wall of the bulla.

In 3 cats the resting potential at the round window was found to be 6.0—7.5 mV relative to a reference point outside the cochlea. This potential increased in response to electrical stimulation of the crossed olivo-cochlear bundle. It was difficult to define the beginning of the effect, as seen in Fig. 23. The estimated latencies in 6 cats have ranged between 7 and 13 msec.

The effect of electrical stimulation of the crossed olivo-cochlear bundle on the resting potential needed some time to reach its maximal value just as the effect on the cochlear microphonic, the action potential and the primary auditory afferents. The times of rise and decay are illustrated in Fig. 24. Using certain stimulating parameters the increase in the resting potential at the round window to electrical stimulation was only transient. This is exemplified by Fig. 25 where the maximal effect is reached within 0.2 sec. The resting potential returned almost to its original level in spite of continued stimulation of the crossed olivo-cochlear fibres. With cessation of stimulation, there seemed to be a small overshoot to a value below the original resting potential.

The change of resting potential was maximally 0.5 mV. It depended on

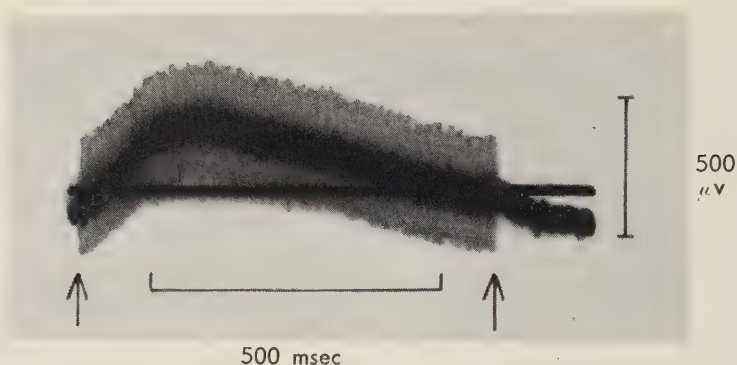


Fig. 25. In this figure is shown the effect of tetanic electrical stimulation of the crossed efferents on the resting potential at the round window. A tetanic burst has been triggered with a short delay relative to the beginning of the sweep. The arrows mark the beginning and the end of the burst. Note that the resting potential returned almost to its original value during continued stimulation of the crossed efferents. With cessation of stimulation there was a slight overshoot of the resting potential. Same cat as in fig. 23 A and fig. 24, same parameters of stimulation, except for duration of burst.

stimulus strength and frequency as did the other effects of crossed olivo-cochlear stimulation.

As a control procedure a shallow short cut was placed 3 mm lateral of the stimulating electrodes in the midline, between these and the recording electrode (in 5 animals). After such a cut electrical stimulation no longer produced any changes of the action potential, cochlear microphonic or resting potential at the round window in animals under Flexedil and/or the middle ear muscles divided (*cf.* GALAMBOS 1956).

The action of strychnine on the response to stimulation of the crossed olivo-cochlear fibres

DESMEDT and MONACO (1960) showed that intravenous injection of strychnine sulphate in doses of 0.05–0.15 mg/kg body weight blocked the effect of electrical stimulation of the olivo-cochlear bundle on the action potential at the round window. Strychnine sulphate applied locally to the one cochlea blocked the ipsilateral response while inhibition of the action potential could still be seen in the other ear. The augmentation of the cochlear microphonic (FEX 1959) could also be blocked by strychnine (DESMEDT and MONACO 1961).

In the present series of experiments strychnine sulphate given intravenously in doses of 0.1–0.15 mg/kg body weight was found to block the effect of olivo-cochlear bundle stimulation on the action potential, cochlear microphonic and resting potential at the round window. A strychnine block that seemed total at one stimulus strength could appear as a partial block at

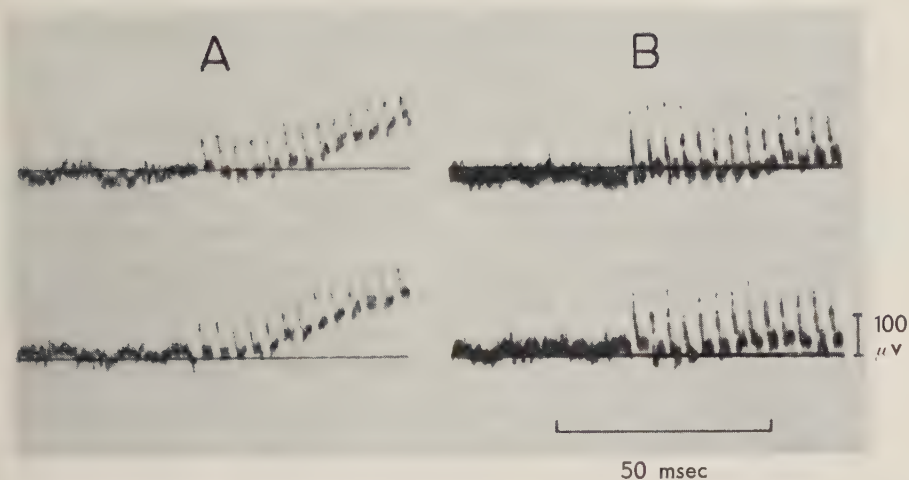


Fig. 26. The blocking effect of strychnine on the increase of the resting potential at the round window from efferent stimulation. Tetanic bursts were triggered with a delay relative to the beginning of the sweeps. In A the result of efferent stimulation is seen before the administration of strychnine. Between A and B strychnine was given intravenously, $130 \mu\text{g/kg}$ body weight. Decerebrate cat with middle ear muscles cut, curarized with Flaxedil. Cat. 28.05.61.

higher stimulus strengths. In Figs. 26 and 27 the change of resting potential in response to electrical stimulation before and during the strychnine block, is illustrated. In Fig. 27 A the maximal effect of the electrical stimulation was reached within 0.2 sec while during the strychnine block in B, the resting potential was still increasing at the end of the 0.25 sec tetanic burst and the change of resting potential at that point was only half of that of Fig. 27 A.

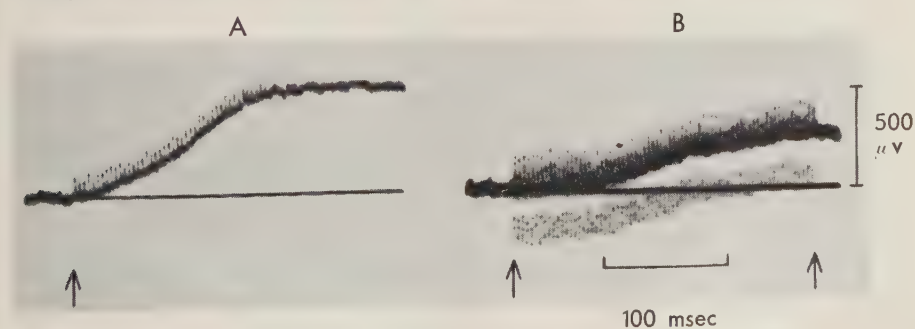


Fig. 27. The blocking effect of strychnine on the resting potential at the round window from efferent stimulation. Tetanic bursts have been triggered with a delay relative to the beginning of the sweeps. The arrows mark the beginning and the end of the burst. In A the result of efferent stimulation is seen before the administration of strychnine. Between A and B strychnine has been given intravenously, $130 \mu\text{g/kg}$ body weight. Same animal and same data as in fig. 26.

DISCUSSION

Basic assumptions

Before the results can be discussed the validity of the basic assumptions underlying the study must be submitted to analysis. These assumptions are that Chapter I deals with crossed olivo-cochlear fibres (see below, A) and that the effects described in Chapters II and III are due to electrical stimulation of crossed olivo-cochlear fibres and of no other fibre systems (see below, B). The experimental situation when the cochlear nerve was stimulated close to the recording electrode in the vestibulo-cochlear anastomosis will also be considered (see below, C).

It has been regarded a necessary and adequate criterion for the identification of a crossed efferent fibre in the vestibulo-cochlear anastomosis, that it could be electrically driven from the floor of the fourth ventricle. The possibility that fibre groups other than the crossed olivo-cochlear ones have been co-stimulated should now be considered in the light of pertinent data on current spread and histological findings.

GALAMBOS (1956) found that if the stimulating electrodes in the floor of the fourth ventricle were moved in the midline more than 2—3 mm from an optimal position, the inhibitory effect on the action potential at the round window was completely lost. This finding has been confirmed in the present work, and several times it has been found that when the stimulating electrodes were moved 1—2 mm in the midline the stimulus strength had to be increased by a factor of five to ten in order to give the effect obtained at the original position. When a shallow cut was placed 3 mm lateral to the stimulus site in the midline, the electrical stimulation no longer produced any changes at the ipsilateral round window in animals deprived of function on the part of the middle ear muscles. Spread to facial fibres innervating the stapedius muscle appeared at stimulus strengths 5—10 times threshold. The facial genua are closer to the stimulating electrodes than any other structures that need be considered.

A. Fibres in the vestibulo-cochlear anastomosis may be 1) unmyelinated autonomic fibres [such fibres have not been found in the anastomosis but have not either been finally excluded (GACEK and RASMUSSEN 1961)], 2) primary auditory afferent fibres (RASMUSSEN 1953a), 3) uncrossed olivo-cochlear fibres (RASMUSSEN 1960) and 4) crossed olivo-cochlear fibres (RASMUSSEN 1946). The question now arises if any fibres other than group 4 possibly might be stimulated in the floor of the fourth ventricle.

ad 1. LORENTE de No (1937) mentions fibres innervating blood vessels in the cochlea of young rats and claims that these fibres reach the inner ear through an anastomosis between the facial and the cochlear nerve; RASMUSSEN (1946) and PORTMANN (1952) both believe that LORENTE de No actually had seen the vestibulo-cochlear anastomosis itself. There is thus no evidence for his hypothetical autonomic fibres to the region stimulated here, as defined in previous paragraph. Neither RASMUSSEN nor PORTMANN has recognized any autonomic fibres in the vestibulo-cochlear anastomosis. Even if such fibres exist it is most unlikely that they could be recorded from by the techniques used in the present work.

ad 2. After destruction of the spiral ganglion a central projection of degenerated primary neurones has been found in the trapezoid body (LEWY and KOBRAK 1936) and the dorsal cochlear nucleus (STOTLER 1961) which are far from the site of the electrodes. It is therefore concluded that in the present work primary auditory afferent fibres cannot have been antidromically stimulated by spread of current.

ad 3. Little has been published on the uncrossed olivo-cochlear fibres. According to RASMUSSEN (1960, *cf.* his Fig. 84) these fibres run from the S-shaped olivary nucleus in a dorso-lateral direction and join the crossed olivo-cochlear fibres from the opposite side lateral to the facial root. These structures are also outside the region that can be reached by current spread. By the electrical midline stimulation uncrossed olivo-cochlear fibres may yet have been activated through unknown internuncial neurones or through the pathway of crossed olivo-cochlear fibres — cochlear nucleus (RASMUSSEN 1960) — superior olive (STOTLER 1953) — uncrossed olivo-cochlear neurones but this possibility seems to be highly speculative, even though it cannot be excluded.

ad 4. Of the different possibilities listed there remains only the crossed efferent bundle. In histological sections from five animals electrode tracts were found in, or close to, crossing bundles that satisfactorily fitted the description given by RASMUSSEN (1946) for the crossed olivo-cochlear fibres.

B. Concerning the effects described in Chapters II and III fibres other than the ones running in the vestibulo-cochlear anastomosis have to be considered. The possibilities are 1) autonomic fibres, 2) primary cochlear afferents, 3) uncrossed efferent cochlear fibres, 4) crossed efferent cochlear fibres, 5) fibres innervating the middle ear muscles.

ad 1. No parasympathetic fibres have ever been shown to travel with the eighth nerve in the modiolus (*cf.* SMITH 1961). Investigators utilizing silver stains (LORENTE de No 1937; SMITH 1951) have not been able to demonstrate any sympathetic fibres accompanying blood vessels peripheral to the modiolus.

Therefore it seems improbable that the effects studied have been due to activation of autonomic fibres running to the organ of Corti.

Autonomic fibres to other parts of the cochlea might change inner ear activity by altering blood supply or as a consequence of secretory processes. In the work of KONISHI, BUTLER and FERNÁNDEZ (1961) will be found the shortest time ever reported for decrease of cochlear potentials (in guinea pig) through interference with the blood supply to the cochlea. After interruption of the arterial blood supply to the cochlea by sectioning the anterior inferior cerebellar artery or by abrupt occlusion, the cochlear potentials started to decrease with a latency of 2–3 sec. The endolymphatic resting potential, the summing potential, the action potential and the cochlear microphonic were studied in that work. The data permit the conclusion that no changes occur during the first second of arterial occlusion.

SMITH (1957), in an electron microscopic study of the structure of the stria vascularis, reported findings suggestive of a secretory function in this organ. Experiences with the sublingual glands may be pertinent for a discussion of whether such a function might be of importance in this connexion (LUNDBERG 1958, also for references). When fibres to the sublingual gland were stimulated secretory potentials were recorded from the gland with latencies of the order of 1 sec and further experimental evidence strongly supported the assumption that these secretory potentials depended upon active ionic transport directly related to secretion.

A critical review of earlier findings of changes of cochlear microphonics induced by stimulation of the sympathetic system is given by BEICKERT, GISELSSON and LÖFSTRÖM (1956) together with results of their own experiments on cats. They found changes that needed minutes to become maximal which could not be produced in animals with the middle ear muscles out of function.

Therefore the participation of autonomic nerve fibres in the production of the early changes described in Chapters II and III can be excluded.

ad 2 and 3. It was concluded above (see A 2) that no primary auditory neurones were within reach of the stimuli applied to the floor of the fourth ventricle. This also holds (see above, A 3) for the uncrossed olivo-cochlear fibres.

ad 4 and 5. The effects under discussion were seen in animals in which the middle ear muscles were put out of function and so could not be due to the action of these muscles.

In A 4 it was inferred that the crossed olivo-cochlear fibres actually were the ones electrically stimulated from the floor of the fourth ventricle and it is now concluded that such stimulation alone produced the effects discussed in Chapter II and Chapter III.

C. The apical part of the cochlear nerve was stimulated electrically and evoked fibre activity recorded in crossed olivo-cochlear fibres in the vestibulo-cochlear anastomosis half a mm from the site of stimulation. The crossed olivo-cochlear fibres could have been activated through 1) current spread from the stimulating electrode, 2) an artificial synapse (GRANIT and SKOGLUND 1945 *a* and *b*) or some kind of ephaptic transmission (*cf.* LLOYD 1942) between primary auditory fibres and crossed efferent fibres, 3) physiological connexions between auditory afferents and the cells of origin of the crossed olivo-cochlear fibres.

ad 1. Some of the evoked potentials had latencies below 1.5 msec and were therefore attributed to current spread. In the other group of potentials the latencies (3.5—10 msec) were too long to be explained in this manner.

ad 2. The latencies, 3.5—10.0 msec of the responses under discussion also presuppose that the site of abnormal transmission must be placed far out in the periphery where postulated lesions in adjoining afferent and efferent fibres necessarily would be sparse. GRANIT and SKOGLUND (1945 *a* and *b*) found fresh sections and low temperature favourable for transmission through artificial synapses. Lesions of afferent and efferent fibres caused by drilling and pulling must have been irregular and were fresh only during the beginning of the procedures. The temperature of the room was not below 30° C.

ad 3. According to RASMUSSEN (1960) the cells of origin of the crossed efferent fibres receive their afferent connexions predominantly from the contralateral cochlear nuclei. The cochlear nerve that was electrically stimulated was thus probably the main pathway for activating the crossed olivo-cochlear neurones under physiological conditions. Whether or not electrical stimulation of the cochlear nerve is a more effective stimulus for the crossed olivo-cochlear fibres than a physiological one is not known.

The latencies for the first impulses in crossed efferent fibres initiated by electrical stimulation of the cochlear nerve were found to be 3.5—10.0 msec. GALAMBOS *et al.* (1959) have reported latencies to sound of 2.5—10.0 msec in the superior olive. The present data are thus compatible with earlier findings.

It is therefore concluded that in the present experiments the crossed olivo-cochlear fibres were activated through their cells of origin when the cochlear nerve was electrically stimulated.

Earlier observations on efferent endings

ENGSTRÖM (1958), utilizing electron microscopic techniques demonstrated in the guinea pig cochlea that there was a marked difference between two kinds of nerve ending. In particular the first row of outer hair cells was found to be

provided with a very large system of nerve terminals filled with rounded granulas. The inference was that these nerve endings actually represented efferent fibres although it seemed surprising that they occurred in such quantities as to presuppose a very considerable amount of terminal splitting. The presence of the two kinds of nerve ending was confirmed by SPOENDLIN (1960) in cats and by IURATO (1961) in rats.

ENGSTRÖM and FERNÁNDEZ (ENGSTRÖM 1961) in a discussion have reported observations to the effect that after sectioning the efferent bundle in the fourth ventricle the number of granulated nerve endings of the outer hair cells was diminished. Whether only the crossed efferent bundle or both the crossed and uncrossed ones were sectioned is not apparent. SMITH (1961) found nerves of small calibre winding themselves around the radial nerve fibres — held to be afferent — and apparently in synaptic contact with them. They sometimes also touch the hair cells. It was suggested that these nerves of small calibre are identical with RASMUSSEN's efferent bundle.

Histological findings thus clearly demonstrate a double innervation of the hair cell region and the studies of the effect of lesions in the fourth ventricle has demonstrated that one of these systems of terminals is efferent. However, it must again be stressed that as yet there has been no differentiation between the two efferent systems.

GISSELSSON (1950) found evidence of acetylcholinesterase in the endolymph of cat and CHURCHILL and SCHUKNECHT (1956) could localize this enzyme to the hair cell region of the organ of Corti and to nerve fibres orientated towards this organ. The acetylcholinesterase in the organ of Corti diminished or disappeared after lesions in the floor of the fourth ventricle (CHURCHILL and SCHUKNECHT 1959; SCHUKNECHT, CHURCHILL and BORAN 1959). They inferred that its disappearance was due to section of the crossed efferent fibres but SCHUKNECHT (1960) added "I was fortunate in that I made my incisions for cutting the olivocochlear bundle in the sulcus limitans far enough laterally to get the homolateral branch which Dr. Rasmussen described yesterday and which I had not known about before."

Further studies on the acetylcholinesterase content of the organ of Corti were made in cats and guinea pigs by VINNIKOV and TITOVA (1958) who showed that one hour of stimulation with loud tones caused a decrease in the concentration of acetylcholinesterase. This fall was localized to the upper part of the cochlea when low-frequency tones were used and in the lower part after high-frequency tone stimulation. DEL-BO and CONTI (1961) found that in guinea pigs younger than ten days acetylcholinesterase was present only in the basal part of the organ of Corti.

A further extension of the possibilities of this technique was made by WER-

SÄLL, HILDING and LUNDQUIST (1961) who completed an electron microscopic study of the distribution of acetylcholinesterase in the organ of Corti in the guinea pig. They stated that it proved impossible to localize the enzyme to the one or the other type of nerve ending because of artefacts from shrinking of the preparations, but in general the distribution of it corresponded to that of the large nerve endings as described by ENGSTRÖM (1958).

A clue to a differentiation between the two efferent systems may be had from the findings of DESMEDT and MONACO (1960, 1961) who demonstrated that strychnine blocked the inhibition of the action potential as well as the augmentation of the cochlear microphonic at the round window, as caused by electrical stimulation of the crossed efferent fibres. Since there has been no reports of strychnine blocking the action of cholinergic fibres in mammals, the findings of DESMEDT and MONACO suggest that the crossed efferent fibres are non-cholinergic. It would follow that the acetylcholinesterase is part of the uncrossed efferent system. This hypothesis may be tested experimentally by techniques of the kind used in this study.

The site of action of the crossed cochlear efferents

In Chapter III it has been demonstrated that the resting potential, the cochlear microphonic and the action potential at the round window all are affected by electrical stimulation of the crossed efferent fibres.

The resting potential at the round window is a reflection of the endolymphatic resting potential (von BÉKÉSY 1952*a*) which originates in the stria vascularis (DAVIS, DEATHERAGE, ROSENBLUT, FERNÁNDEZ, KIMURA and SMITH 1958; MISRAHY, DE JONGE, SHINABARGER and ARNOLD 1958; TASAKI and SPYROPOULOS 1959). The site of the cochlear microphonic of the inner ear was placed by von BÉKÉSY (1952*b*) to the organ of Corti. TASAKI, DAVIS and ELDREDGE (1954) using the technique of von BÉKÉSY claimed that the hair cells are the source of the microphonic potential. Synchronous firing of a large number of nerve impulses is the accepted explanation of the action potential at the round window (see DAVIS 1957).

Present concepts of the mechanism by which acoustical energy is transduced into nerve impulses are summarized by DAVIS (1961) in the following words: "The stria vascularis of the cochlea is such a 'battery' (fig. 7) and it hyperpolarizes the hair cells. The receptor potentials of the ear are the cochlear microphonic and the summing potential. Both potentials derive energy from the hair cells and are controlled in the hair cells by a mechanical action, but they also draw additional energy from the stria vascularis."

It has been argued above that the stria vascularis as a secretory organ cannot cause the changes under discussion. The observed increase of the

resting potential at the round window is then probably due to a decreased impedance of the organ of Corti to the current driven by the endocochlear potential.

It was shown above that the cochlear microphonic is increased by the efferent stimulation while the action potential at the round window is decreased. From the study of primary auditory afferents it was concluded that no facilitatory processes were concealed behind the decrease.

Present knowledge concerning the relations of the resting potential, the cochlear microphonic and the action potential at the round window permit no conclusion as to whether or not the observed changes of these potentials actually are causally related. It is impossible at this stage to conclude anything more definite concerning which structures in the organ of Corti are affected by the efferent activity. In general, however, the present findings are fully compatible with the histological suggestions according to which the crossed efferents terminate on hair cells.

There seems to be a discrepancy between the effects of efferent stimulation on the receptor potential and on the action potential inasmuch as the former increases, the latter diminishes. Further studies of the effects of efferent stimulation on all the cochlear potentials, including the summation potential, and on the primary afferents, may solve the discrepancy observed. Such studies may also contribute to a deeper understanding of the mechanisms whereby sound is translated into nerve impulses.

Activity and function of the crossed cochlear efferents

Although the crossed olivo-cochlear neurones were adequately activated by the cochlear nerves, their firing pattern did not closely follow that of the primary and secondary auditory neurones. The differences in firing pattern and the long latencies indicate that the input to the crossed olivo-cochlear neurones would be integrated over a considerable time. During these integrative processes irregularities in the input activity are smoothed out.

A reproducible threshold of sound pressure at a best "frequency" and a well defined band width of tone frequency at different pressure levels, were some of the features common to afferents and efferents.

Crossed olivo-cochlear fibres in the basal fascicle of the vestibulo-cochlear anastomosis generally responded to higher tone frequencies than did the fibres in the apical fascicle (p. 25). This leads to the conclusion that sound of high tone frequencies to one ear suppresses the response to high-frequency sound in the other ear. It may well be that this is an expression of a general principle according to which the nerve endings of the crossed efferent fibres are orderly arranged in a spatial sequence similar to that of the afferent nerve endings in

the cochlea so that afferents and efferents from homotopic cochlear points in opposite cochleas are connected. The selective depletion of acetylcholinesterase — in the basal part of the cochlea after stimulation with high tone frequencies, in the apical part after low tones (VINNIKOV and TITOVA 1958) — is also suggestive of such an orderly arrangement of efferent nerve endings, presumably those of the uncrossed efferent system (see above for discussion, p. 56).

For the understanding of the function of the crossed efferent system it should be kept in mind that the superior olive is an auditory relay on to which afferent auditory neurones converge most of which are second order neurones (STOTLER 1953). ROSENZWEIG and ANON (1955) have found binaural interaction of clicks in the superior olive, as studied with macroelectrodes in acute experiments on cats.

The experiments reported here were not ideal for analyzing this bilateral representation since the ear providing the predominant afferent inflow (*cf.* RASMUSSEN 1960) to the crossed efferents under study had been put out of function as part of the preparation for the experiment. In spite of these experimental shortcomings it could be demonstrated that sound stimulation of the ear ipsilateral to the superior olive increased the responsiveness of these neurones to electrical stimulation of the contralateral auditory nerve. This result seems to be in harmony with those of GALAMBOS *et al.* (1959) although the experimental conditions differed. They suggested further that nucleus accessorius contains a class of neurones concerned chiefly with preserving the exact time of arrival of stimuli at the cochlea. This is of interest because dorsal cells in the region of the rostral third of nucleus accessorius give origin to the crossed efferent cochlear fibres (GALAMBOS *et al.* 1959).

It appears from the results of Chapters I and II and the foregoing discussion on the significance of electrical stimulation of the cochlear nerve that the crossed efferents form part of a closed feedback loop (inner ear — cochlear nucleus — contralateral superior olive — crossed efferents back to ear).

The results of Chapters II and III may represent sub-maximal effects since they were derived from experiments with the proposed feedback loop closed, with one exception, cat 08.12.61. in which the crossed efferents were cut above the stimulating electrode in the fourth ventricle.

It might be of interest to compare the crossed cochlear feedback system with that involving the middle ear muscles. It is well-known that the reflex muscle contraction to sound is largely (*e.g.* in cat, *cf.* SIMMONS 1959), or exclusively (in man, *cf.* KLOCKHOFF 1961), due to the stapedius. The reflex arc of this muscle is not exactly known but probably runs in auditory afferents to the superior olive, then to the facial nucleus and further on to fibres

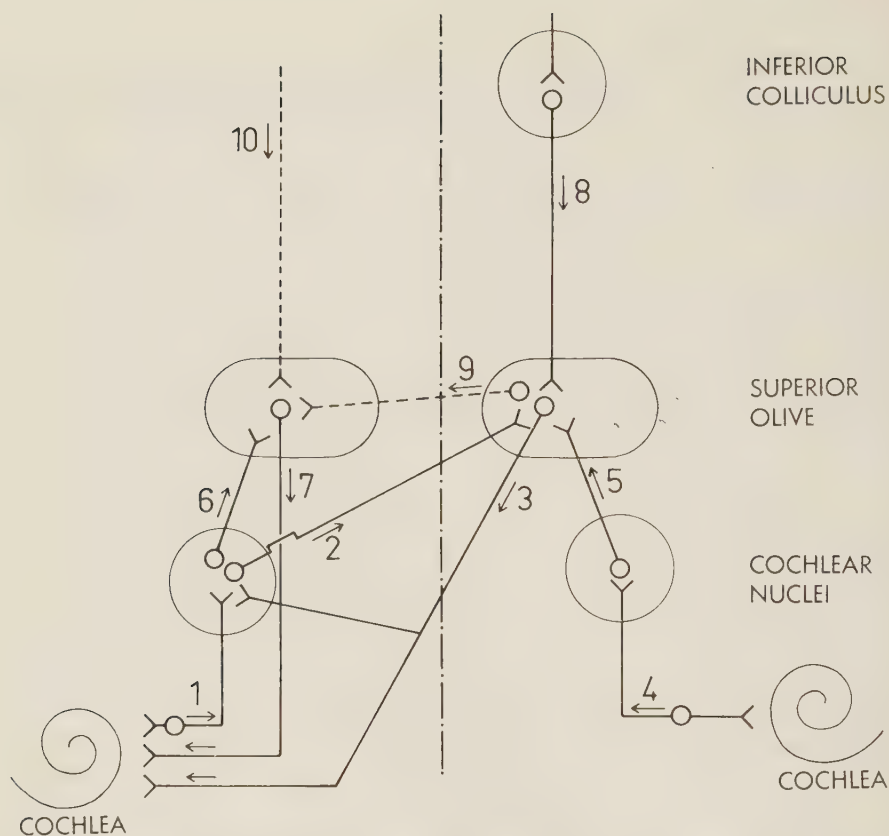


Fig. 28. Diagram illustrating the feedback system of the left cochlea. Only primary afferents shown for the right cochlea, although every connexion depicted has its contralateral homotopic counterpart. The terms "ipsilateral" and "contralateral" refer to the left ear. 1, primary auditory afferents from cochlea to ipsilateral cochlear nuclei; 2, connexions from ipsilateral cochlear nuclei crossing to the contralateral superior olive; 3, the efferent tract of olivo-cochlear fibres from the contralateral superior olive crossing to the cochlea. — Connexions 1—2—3 constitute the closed feedback loop. 4, primary auditory fibres from the contralateral cochlea to the contralateral cochlear nuclei; 5, connexions from the contralateral cochlear nuclei to the contralateral superior olive. Through connexions 4—5 the closed feedback loop of connexions 1—2—3 can be influenced. — 6, connexion from the ipsilateral cochlear nuclei to the ipsilateral superior olive; 7, the uncrossed efferent connexion from the ipsilateral superior olive to the cochlea. Connexions 1—6—7 may constitute a closed feedback loop. — 8, connexions from the contralateral inferior colliculus to the contralateral superior olive. Tract 8 may influence activity in the closed feedback loop of connexions 1—2—3. — 9, proposed connexions from the contralateral superior olive to the uncrossed efferent neurones, which would make it possible for input to the contralateral ear to influence activity in the proposed closed feedback loop of connexions 1—6—7. No direct connexions between the contralateral cochlear nuclei and the ipsilateral uncrossed efferent neurones are known. — 10, proposed connexions from nuclei central to the superior olive to the uncrossed efferent neurones, which would enable central control of the proposed feedback loop of connexions 1—6—7.

destined for the stapedius (*cf.* JEPSEN 1955). It has been shown by MÖLLER (1961) by impedance measurements on man that the stapedius reflex nearly always was more sensitive to ipsilateral stimulation. The sensitivity was defined by him as the intensity required for a definite percentage of the maximum impedance change.

Latencies from first cochlear potentials to the earliest muscle potentials were for the stapedius 6 ± 0.5 msec and for the tensor tympani 7 ± 0.7 msec in decerebrated cats (ELIASSON and GISSELSSON 1955). WERSÄLL (1958) found in rabbits under pentobarbitone that the attainment of 50 per cent of the final reflex tension was reached for the stapedius muscle in 40—151 msec and for the tensor tympanic muscle in 44—174 msec. This time depended but little on sound intensity. SIMMONS (1959) who monitored round window activity through chronically implanted electrodes in awake cats reported attenuation of up to 30 db of the cochlear microphonic through the action of the middle ear muscles.

Comparing available figures, *i. e.* those given now for latency, course of development and attenuation of afferent input by the reflex route and corresponding ones presented above for stimulation by the efferent fibres, it is of some interest to note that they are of the same general order.

Recent experimental results (KLOCKHOFF and ANDERSON 1960; KLOCKHOFF 1961; SIMMONS, GALAMBOS and RUPERT 1959; HUGELIN, DUMONT and PAILLAS 1960) show that the control of the middle ear muscles is not directed according to a rigid pattern through a simple feedback loop but that setting mechanisms may to a large extent regulate their activity.

Recently several experiments besides those already referred to have been performed in an effort to trace centrifugal effects on auditory activity at the level of the inner ear. Decrease of the action potential component at the round window as an expression of habituation has been found in cats with chronically implanted electrodes (AL'TMAN 1960) as well as in relation to the development of an orientation reaction (MARUSEVA 1961). The findings were also obtained in cats in which the middle ear muscles had been cut and so were interpreted as true centrifugal effects. GALAMBOS (1960) reported a series of negative findings in experiments of a similar type.

GALAMBOS (1960) also failed to find evidence of crossed olivo-cochlear activation when shocks were applied to the region of the brachium of the inferior colliculus. These findings are most interesting because RASMUSSEN (1953*b*, 1955) has reported descending degeneration following lesions of the inferior colliculus and/or the dorsal nucleus of the lateral lemniscus. This degeneration projects predominantly to that region of the superior olive which previously was found to be a site of origin of the crossed olivo-cochlear fibres.

DESMEDT (1960, foot note p. 155) however, found it possible to record a decrease of the evoked action potentials both at the round window and in the cochlear nucleus, when curarized cats were stimulated in this region. It is not known if the effect at the round window was due to the crossed or the uncrossed bundle, but the data favour the former alternative.

The present study has thus given the following main new results. Efferent fibres in the cochlea from the contralateral superior olive are activated by the afferent fibres from the same cochlea. They thus form part of a feedback loop within the auditory system. The activity in this loop is influenced by input from the contralateral ear. The action of the crossed efferents on the primary afferents is purely inhibitory and takes place in the organ of Corti simultaneously with an increase of the resting potential and with an increase of the cochlear microphonic at the round window.

In Fig. 28 the feedback control of the auditory input has been sketched for one side. A closed loop is formed by primary auditory afferents (1) from the cochlea, secondary auditory fibres (2) crossing to the contralateral superior olive and the crossed olivo-cochlear fibres (3) returning to the cochlea. The feedback control of the auditory input through this loop is influenced by afferents (4—5) from the contralateral ear to the contralateral superior olive. The two symmetrical closed loops controlling each ear (only one side sketched) are thus interconnected and, very probably, together with the uncrossed olivo-cochlear efferents (7) form a complex feedback control mechanism. This system is very likely regulated from higher centres. The connexion (8) between the region of the inferior colliculus and the cells of origin of the crossed cochlear efferents may be one of the possible pathways for such a centrifugal control.

SUMMARY

In the first part of this study a technique has been developed for recording from single crossed olivo-cochlear efferent fibres in the vestibular-cochlear anastomosis; decerebrate cats have been used in which the auditory function of the left ear has been destroyed as part of the preparation.

Crossed olivo-cochlear fibres could be activated by sound applied to the contralateral ear. The activity in the neurones has been analyzed with respect to resting activity, firing pattern, "best frequency", tone frequency band width, and latency.

The crossed olivo-cochlear fibres responded as a rule with a low, regular firing rate without initial bursts. This indicated that the input to the crossed efferents would be integrated over a considerable time.

Crossed olivo-cochlear fibres in the basal fascicle of the vestibulo-cochlear anastomosis generally responded to higher tone frequencies presented to the contralateral ear than did the fibres in the apical fascicle. This may be an expression of a general principle according to which afferents and efferents from homotopic cochlear points in opposite cochleas are connected.

Crossed efferent fibres were also activated by electrical stimulation of the ipsilateral auditory nerve. This activity was analyzed with respect to latency and firing pattern.

Crossed efferents in the cochlea showed increased responsiveness to electrical stimulation of the ipsilateral auditory nerve when sound was presented to the contralateral ear.

In the second part of the study a technique has been described for recording from single primary auditory afferents. Decerebrate cats with both cochleas intact were used. A histological criterion for the identification of primary auditory neurones has been introduced. The effect of electrical stimulation of the crossed efferents on primary auditory neurones was studied. Such stimulation inhibited resting activity as well as sound evoked activity in the primary auditory neurones. Neither facilitation nor rebound after inhibition was seen.

In the third part of the study the effect of electrical stimulation on cochlear potentials was studied with macroelectrodes at the round window. Such stimulation increased the resting potential, increased the cochlear microphonic (seen earlier) and diminished the action potential (GALAMBOS 1956). It was concluded that the effect on the resting potential was due to a decreased impedance of the organ of Corti to the current driven by the endocochlear potential.

The results have been further discussed in the light of current knowledge of the double innervation of the organ of Corti.

The results permit the conclusion that auditory afferent fibres and the crossed efferent fibres together form a closed feedback loop within the auditory system. The efferents of this loop are also activated from the contralateral ear. These main new results lead to the final conclusion that the two crossed olivo-cochlear bundles are part of a complex feedback control mechanism.

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REFERENCES

- ALEXANDER, G. und H. OBERSTEINER, Das Verhalten des normalen Nervus cochlearis im Meatus auditorius internus. *Z. Hals-, Nas.- u. Ohrenheilk.* 1908. 55. 78—91.
- ALTMAN, I. A., Electrophysiological examination of different parts of the auditory system in the cat during sustained rhythmical stimulation. *Sechenov Physiol. J. USSR.* 1960. 46. 617—629. (*Fiziol. zh. SSSR.* 1960. 46. 526—536.)
- BACH-Y-RITA, G., H. BRUST-CARMONA, J. PEÑALOZA-ROJAS and R. HERNÁNDEZ-PEÓN, Absence of para-auditory descending influences on the cochlear nucleus during distraction and habituation. *Acta neurol. latinoamer.* 1961. 7. 73—81.
- BEICKERT, P., L. GISSELSSON und B. LÖFSTRÖM, Der Einfluss des sympathischen Nervensystems auf das Innenohr. *Archiv Ohren usw. Heilk. u. Z. Hals- usw. Heilk.* 1956. 168. 495—507.
- BÉKÉSY, G. v., D-C potentials and energy balance of the cochlear partition. *J. acoust. Soc. Amer.* 1950. 22. 576—582.
- BÉKÉSY, G. v., D-C potentials inside the cochlear partition. *J. acoust. Soc. Amer.* 1952*a*. 24. 72—76.
- BÉKÉSY, G. v., Gross localization of the place of origin of the cochlear microphonics. *J. acoust. Soc. Amer.* 1952*b*. 24. 399—409.
- BROWN, K. T. and K. TASAKI, Localization of electrical activity in the cat retina by an electrode marking method. *J. Physiol.* 1961. 158. 281—295.
- CHURCHILL, J. A. and H. F. SCHUKNECHT, Acetylcholinesterase activity in the cochlea. *Laryngoscope.* 1956. 66. 1—15.
- CHURCHILL, J. A. and H. F. SCHUKNECHT, The relationship of acetylcholinesterase in the cochlea to the olivocochlear bundle. *Henry Ford hosp. med. bull.* 1959. 7. 202—210.
- CONTU, P., Observations and considerations about the cochlear innervation of the cat. *Laryngoscope.* 1958. 68. 586—595.
- CONTU, P., R. M. NEVES PINTO and M. D. S. L. SANTOS, Considerações sobre a anatomia do ramo coclear do nervo acústico (no *Felis Domestica*). *An. Fac. med. univ. Recife.* 1956. 16. 271—276.
- DAVIS, H., Biophysics and physiology of the inner ear. *Physiol. Rev.* 1957. 37. 1—49.
- DAVIS, H., Some principles of sensory receptor action. *Physiol. Rev.* 1961. 41. 391—416.
- DAVIS, H., B. H. DEATHERAGE, B. ROSENBLUT, C. FERNÁNDEZ, R. KIMURA and C. A. SMITH, Modification of cochlear potentials produced by streptomycin poisoning and by extensive venous obstruction. *Laryngoscope.* 1958. 68. 596—627.
- DEL-BO, M. e A. CONTI, Distribuzione dell'acetilcolinesterasi nella lamina spirale della coclea di cavia. *Arch. ital. Otol. Rinol. Laringol.* 1961. 72. 44—55.
- DESMEDT, J. E., Neurophysiological mechanisms controlling acoustic input. In *Neural mechanisms of the auditory and vestibular systems*. Chap. 11. Ed. Rasmussen, G. L. and W. F. Windle. Springfield: C. C. Thomas. 1960. pp. 152—164.
- DESMEDT, J. E. et P. MONACO, Suppression par la strychnine de l'effet inhibiteur centrifuge exercé par le faisceau olivo-cochléaire. *Arch. int. Pharmacodyn.* 1960. 129. 244—248.
- DESMEDT, J. E. and P. MONACO, Mode of action of the efferent olivo-cochlear bundle on the inner ear. *Nature.* 1961. 192. 1263—1265.
- ELIASSON, S. and L. GISSELSSON, Electromyographic studies of the middle ear muscles of the cat. *Electroenceph. clin. Neurophysiol.* 1955. 7. 399—406.
- ENGSTRÖM, H., On the double innervation of the sensory epithelia of the inner ear. *Acta oto-laryng., Stockh.* 1958. 49. 109—118.

- ENGSTRÖM, H., In Discussion to C. A. Smith: Innervation of cochlea. Trans. Amer. Otol. Soc. 1961. 44. 58—60.
- FEX, J., Augmentation of the cochlear microphonics by stimulation of efferent fibres to cochlea. Acta oto-laryng., Stockh. 1959. 50. 540—541.
- GACEK, R. R., Efferent component of the vestibular nerve. In *Neural mechanisms of the auditory and vestibular systems*. Chap. 20. Ed. Rasmussen, G. L. and W. F. Windle. Springfield: C. C. Thomas. 1960. pp. 276—284.
- GACEK, R. R. and G. L. RASMUSSEN, Fiber analysis of the statoacoustic nerve of guinea pig, cat and monkey. Anat. Rec. 1961. 139. 455—463.
- GALAMBOS, R., Suppression of auditory nerve activity by stimulation of efferent fibers to cochlea. J. Neurophysiol. 1956. 19. 424—437.
- GALAMBOS, R., Studies of the auditory system with implanted electrodes. In *Neural mechanisms of the auditory and vestibular systems*. Chap. 10. Ed. Rasmussen, G. L. and W. F. Windle. Springfield: C. C. Thomas. 1960. pp. 137—151.
- GALAMBOS, R. and H. DAVIS, The response of single auditory-nerve fibers to acoustic stimulation. J. Neurophysiol. 1943. 6. 39—57.
- GALAMBOS, R. and H. DAVIS, Action potentials from single auditory-nerve fibers? Science. 1948. 108. 513.
- GALAMBOS, R., J. SCHWARTZKOPFF and A. RUPERT, Microelectrode study of superior olive nuclei. Amer. J. Physiol. 1959. 197. 527—536.
- GISSELSSON, L., Experimental investigation into the problem of humoral transmission in the cochlea. Acta oto-laryng., Stockh. 1950. suppl. 82. pp. 78.
- GRANIT, R., *Receptors and sensory perception*. New Haven: Yale Univ. Press. 1955. pp. 369.
- GRANIT, R. and C. R. SKOGLUND, The effect of temperature on the artificial synapse formed by the cut end of the mammalian nerve. J. Neurophysiol. 1945a. 8. 211—217.
- GRANIT, R. and C. R. SKOGLUND, Facilitation, inhibition and depression at the “artificial synapse” formed by the cut end of a mammalian nerve. J. Physiol. 1945b. 103. 435—448.
- GREEN, J. D., A simple microelectrode for recording from the central nervous system. Nature. 1958. 182. 962.
- HAGBARTH, K.-E., Centrifugal mechanisms of sensory control. Ergebn. Biol. 1960. 22. 47—66.
- HAGBARTH, K.-E. and J. FEX, Centrifugal influences on single unit activity in spinal sensory paths. J. Neurophysiol. 1959. 22. 321—338.
- HUGELIN, A., S. DUMONT and N. PAILLAS, Formation reticulaire et transmission des informations auditives au niveau de l'oreille moyenne et des voies acoustiques central. Electroenceph. clin. Neurophysiol. 1960. 12. 797—818.
- IURATO, S., Submicroscopic structure of the membranous labyrinth. 2. The epithelium of Corti's organ. Z. Zellforsch. 1961. 53. 259—298.
- JAVID, M., Urea — new use of an old agent. Reduction of intracranial and intraocular pressure. Surg. clin. N. Amer. 1958. 907—928.
- JAVID, M. and P. SETTLAGE, Effect of urea on cerebrospinal fluid pressure in human subjects. J. Amer. med. Ass. 1956. 160. 943—949.
- JEPSEN, O., Studies on the acoustic stapedius reflex in man. Thesis. Aarhus: Universitets forlaget. 1955. pp. 118.
- KATSUKI, Y., T. SUMI, H. UCHIYAMA and T. WATANABE, Electric responses of auditory neurons in cat to sound stimulation. J. Neurophysiol. 1958. 21. 569—588.
- KLOCKHOFF, I., Middle ear muscle reflexes in man. Acta oto-laryng., Stockh. 1961. suppl. 164. pp. 92.
- KLOCKHOFF, I. and H. ANDERSON, Reflex activity in the tensor tympani muscle recorded in man. Acta oto-laryng., Stockh. 1960. 51. 184—188.

- KONISHI, T., R. A. BUTLER and C. FERNÁNDEZ, Effect of anoxia on cochlear potentials. J. acoust. Soc. Amer. 1961. 33. 349—356.
- LEWY, F. H. and H. G. KOBRACK, The neural projection of the cochlear spirals on the primary acoustic centers. Arch. Neurol. Psychiat., Chicago. 1936. 35. 839—852.
- LLOYD, D. P. C., Stimulation of peripheral nerve terminations by active muscle. J. Neurophysiol. 1942. 5. 153—165.
- LORENTE DE NÓ, R., The sensory endings in the cochlea. Trans. Amer. Otol. Soc. 1937. 27. 86—90.
- LUNDBERG, A., Electrophysiology of salivary glands. Physiol. Rev. 1958. 38. 21—40.
- MARUSEVA, A. M., The electrophysiological expression of changes in the function of the auditory system associated with the occurrence of an orienting reaction. Sechenov Physiol. J. USSR. 1961. 47. 599—608. (Fiziol. zh. SSSR. 1961. 47. 542—550.)
- MISRAHY, G. A., B. R. DE JONGE, E. W. SHINABARGER and J. E. ARNOLD, Effects of localized hypoxia on the electrophysiological activity of cochlea of the guinea pig. J. acoust. Soc. Amer. 1958. 30. 705—709.
- MÖLLER, A., Bilateral contraction of the tympanic muscles in man. Report from The Speech Transmission Laboratory, The Royal Institute of Technology, Stockholm, Sweden. 1961. 18. pp. 51.
- OORT, H., Ueber die Verästelung des Nervus octavus bei Säugetieren. (Modell des Utriculus und Sacculus des Kaninchens.) Anat. Anz. 1918. 51. 272—280.
- PORTMANN, M.-R., Les fibres nerveuses efférents cochléaires. Thèse pour le doctorat en médecine. Faculté de Médecine et de Pharmacie. Bordeaux 1952. 108. pp. 66.
- RAMÓN CAJAL, S., *Histologie du système nerveux de l'homme & des vertébrés*. Madrid 1952. t.I. pp. 986.
- RASMUSSEN, G. L., The olivary peduncle and other fiber projections of the superior olivary complex. J. comp. Neurol. 1946. 84. 141—220.
- RASMUSSEN, G. L., Further observations of the efferent cochlear bundle. J. comp. Neurol. 1953a. 99. 61—74.
- RASMUSSEN, G. L., Recurrent or "feed back" connections of the auditory system of the cat. Anat. Rec. 1953b. 115. 361.
- RASMUSSEN, G. L., Descending or "feed-back" connections of auditory system of the cat. Amer. J. Physiol. 1955. 183. 653.
- RASMUSSEN, G. L., Efferent fibers of the cochlear nerve and cochlear nucleus. In *Neural mechanisms of the auditory and vestibular systems*. Chap. 8 Ed. Rasmussen, G. L. and W. F. Windle. Springfield: C. C. Thomas. 1960. pp. 105—115.
- RASMUSSEN, G. L. and R. R. GACEK, Concerning the question of an efferent fiber component of the vestibular nerve of the cat. Anat. Rec. 1958. 130. 361—362.
- ROSE, J. E., R. GALAMBOS and J. H. HUGHES, Microelectrode studies of the cochlear nuclei of the cat. Bull. Johns Hopkins Hosp. 1959. 104. 211—251.
- ROSENZWEIG, M. R. and A. H. ANON, Binaural interaction in the medulla of the cat. Experientia. 1955. 11. 498—500.
- RUBEN, R. J. and J. SEKULA, Inhibition of central auditory response. Science. 1960. 131. 163.
- SCHUKNECHT, H. F., In Discussion of anatomy and physiology of peripheral auditory mechanisms. In *Neural mechanisms of the auditory and vestibular systems*. Chap. 7. Ed. Rasmussen, G. L. and W. F. Windle. Springfield: C. C. Thomas. 1960. pp. 91—104.
- SCHUKNECHT, H. F., J. A. CHURCHILL and R. BORAN, The localization of acetylcholinesterase in the cochlea. Arch. Otolaryng., Chicago. 1959. 69. 549—559.
- SIMMONS, F. B., Middle ear muscle activity at moderate sound levels. Ann. Otol., etc., St Louis. 1959. 68. 1126—1143.
- SIMMONS, F. B., R. GALAMBOS and A. RUPERT, Conditioned response of middle ear muscles. Amer. J. Physiol. 1959. 197. 537—538.

- SMITH, C., Capillary areas of the cochlea in the guinea pig. *Laryngoscope*. 1951. 61. 1073—1095.
- SMITH, C., Structure of the stria vascularis and the spiral prominence. *Ann. Otol., etc., St Louis*. 1957. 66. 521—536.
- SMITH, C. A., Innervation pattern of the cochlea. *Ann. Otol., etc., St Louis* 1961. 70. 504—527.
- SPOENDLIN, H., Submikroskopische Strukturen im Cortischen Organ der Katze. *Acta otolaryng. Stockh.* 1960. 52. 111—130.
- STARK, L., Stability, oscillations, and noise in the human pupil servomechanism. *Proc. Inst. Radio Engrs, N. Y.* 1959. 47. 1925—1939.
- STOTLER, W. A., An experimental study of the cells and connections of the superior olivary complex of the cat. *J. comp. Neurol.* 1953. 98. 401—431.
- STOTLER, W. A., Connections of the cochlear nuclei in the cat. *Anat. Rec.* 1961. 139. 276.
- TARLOV, I. M., Structure of the nerve root. I. Nature of the junction between the central and the peripheral nervous system. *Arch. Neurol. Psychiat., Chicago*. 1937. 37. 555—583.
- TASAKI, I., Nerve impulses in individual auditory nerve fibers of guinea pig. *J. Neurophysiol.* 1954. 17. 97—122.
- TASAKI, I. and H. DAVIS, Electric responses of individual nerve elements in cochlear nucleus to sound stimulation (guinea pig). *J. Neurophysiol.* 1955. 18. 151—158.
- TASAKI, I., H. DAVIS and D. H. ELDREDGE, Exploration of cochlear potentials in guinea pig with a microelectrode. *J. acoust. Soc. Amer.* 1954. 26. 765—773.
- TASAKI, I. and C. S. SPYROPOULOS, Stria vascularis as source of endocochlear potential. *J. Neurophysiol.* 1959. 22. 149—155.
- VINNIKOV, IA, A. and L. K. TITOVA, Distribution of specific acetylcholinesterase in Corti's organ with and without sound stimulation. *Proc. Acad. Sciences USSR. Biol. sci. sect.* 1958. 119. 144—148.
- WERSÄLL, J., D. HILDING and P.-G. LUNDQUIST, Ultrastruktur und Innervation der cochlearen Haarzellen. *Arch. Ohren- usw. Heilk. Z. Hals- usw. Heilk.* 1961. 178. 106—126.
- WERSÄLL, R., The tympanic muscles and their reflexes. *Acta oto-laryng., Stockh.* 1958. suppl. 139. pp. 112.

